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THE STRUCTURE OF THE SPINAL CORD OF THE OSTRICH.

BY

GEORGE L. STREETER, M. D.

Assistant in Anatomy, The Johns Hopkins University, Baltimore.

From the Dr. Senckenberg Anatomie, Frankfort-on-Main.

WITH 6 TEXT FIGURES.

It is related by Herodian how the Kaiser Commodus beheaded ostriches and then watched them with delight and wonder as they continued running about the amphitheater, apparently to no great extent inconvenienced by the loss of their heads. That which served Kaiser Commodus as barbarous amusement frames itself for us into an interesting anatomical problem, and calls to mind a similar phenomenon so often observed among the domestic fowls. What is, then, this arrangement of the nervous elements of the spinal cord of a bird that enables it to functionate so completely after separation from the higher centers?

Our present knowledge and methods do not suffice for a complete explanation of this problem, but we can lead the way toward a future solution if we study out what can be learned at present concerning the histology of the bird spinal cord. In this sense, under the suggestion and guidance of Professor Edinger, I have undertaken the investigation of the structure of the spinal cord of the ostrich (*Struthio camelus*). This, beyond all other birds, distinguishes itself by the great length of its spinal cord, and, in comparison with the brain, its great size.

In the literature frequent reference is [illegible]

ERRATA TO VOL. III.

MAKE THE FOLLOWING CORRECTIONS IN

DR. R. G. HARRISON'S PAPER ON: "AN EXPERIMENTAL STUDY OF THE RELATION OF THE NERVOUS SYSTEM TO THE DEVELOPING MUSCULATURE IN THE EMBRYO OF THE FROG;"

Page 209, line 14. Instead of "substances" read "substance"; instead of "nerve" read "muscle."

Page 218, line 1. After "on" insert "the development of."

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In the literature, frequent reference is made to the spinal cord of birds. As early as 1868 *Stieda** presents what could be seen in unstained preparations. He gives a review of the previous literature reaching back to *Steno,* 1667, and *Perrault,* 1699. All of the older investigators of the spinal cord, such as *Stilling* and *Clarke,* have also studied more or less that of the bird, but it is the above mentioned work of Stieda that gave us first a clear and complete description. Of the more recent anatomists, mention is to be made of the works of *Gadow*[1] and *Kölliker.*[2] A number of investigations have been made which were limi-

* Stieda, Studien über das centrale Nervensystem der Vögel und Säugethiere.

[1] Gadow, Bronn's Klassen und Ordnungen des Thierreiches, Bd. 6, p. 406.

[2] Kölliker, Gewebelehre, 1896.

ted to various parts of the cord, as, for example, the study of *Duval*[3] concerning the Sinus rhomboidalis, and an experimental work of *Friedländer*[4] on the fibre tracts. To these may be added also the works of *Singer, Münzer,* and others who devoted themselves more particularly to the brain. It is, further, not to be forgotten that the studies of *Retzius, Ramon-y-Cajal, van Gehuchten,* and *v. Lenhossek* concerning the nerve-cells and fibres of the spinal cord in Golgi preparations were carried out largely on the chick. No attention, however, seems to have been directed toward the spinal cord of the ostrich. A cross-section, apparently of the thoracic region, is pictured by *Edinger,*[5] but is not otherwise described.

The material on which this study is based consisted of three ostrich spinal cords taken from the neurological collection of the Anatomie. Two were practically intact; the third had been cut into segments. All three had been hardened in formol. After the macroscopic examination was completed, series of transverse sections were made in all segments. Unbroken sagittal and fronto-longitudinal series were prepared through three segments of the lumbar enlargement, and a fronto-longitudinal series of one segment in the cervical region. Sections were also prepared of a decalcified vertebra showing the cord *in situ* with its membranes, the nerve roots, and spinal ganglia. Where other than the usual stains were used they are specified in the text.

The Meninges.

The cord is supported in the vertebral canal by a connective tissue sheath which, like that in mammals, may be described as consisting of three separate membranes or envelopes. In order, from within outwards, they are the pia, arachnoidea, and dura. These structures are represented in Fig. 1.

Of the three envelopes the *dura* is by far the strongest. It is this that forms the tough fibrous sheath surrounding the cord, which one sees on the removal of the latter from the vertebral canal. It consists of a membrane .011 to .012 mm. thick, made of thickly-lying coarse fibres, and contains no blood-vessels. Outside the dura is a connective tissue layer which lines the vertebral canal, and forms the periosteum of the vertebræ. This, having the same histological character, may be described

[3] Duval, Recherchés sur le Sinus Rhomboidal des Oiseaux, Journ. de l'Anat. et de la Phys., 1877.

[4] Friedländer, Untersuch. über das Rückenmark und das Kleinhirn der Vögel, Neurolog. Centrabl., 1898.

[5] Edinger, Nervöse Centralorgane, 1900, p. 76.

as belonging to the dura, and as forming its outer layer. The cleft between the two, the *epidural cavity,* is bridged over by loose strands of tissue supporting a plexus of blood-vessels.

More or less adherent to the inner surface of the dura is the *arachnoidea.* Whether or not this, in the fresh state, is completely adherent to and possibly a part of the dura, could not be decided, as all the material used in this study had been through a prolonged hardening in formol. In the preparations at irregular intervals, they were still adherent, but in the greater part there was a separation of the two membranes, having more the appearance of an artificial tearing apart, or shrinkage forma-

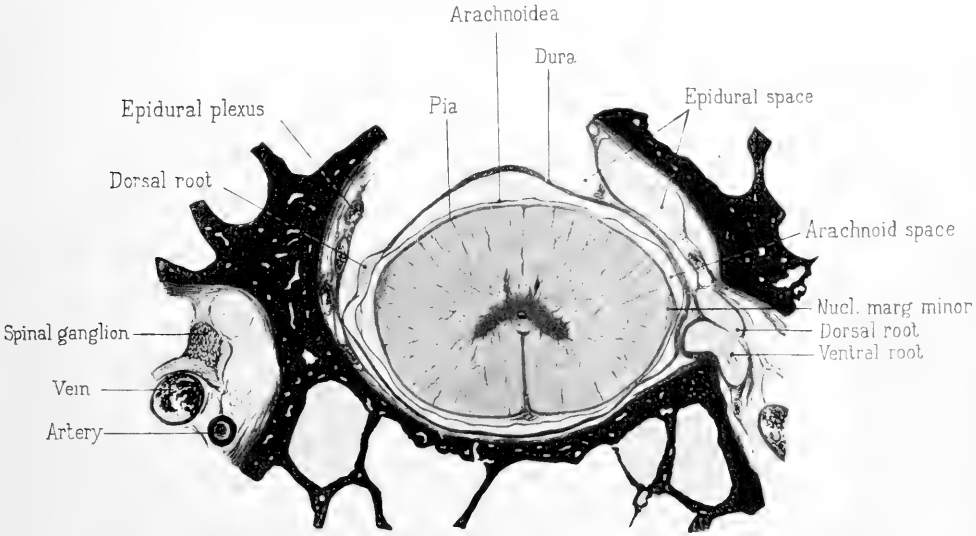

Fig. 1. Cross-section through the 4th cervical vertebra of the ostrich, showing the spinal cord and its membranes. One side is drawn at a point somewhat higher than the other. Enlargement × 6.

tion, than a natural cleft. In the space thus formed, there was no trace of serum, blood-cells, or other tissue. In cross-sections the arachnoidea shows itself as a delicate, thickly nucleated membrane, connected from its inner surface with the pia by a network of fine strands which form a meshwork of lymph spaces for the cerebro-spinal fluid, *subarachnoideal cavity.*

The rootlets forming the ventral and dorsal nerve roots take their course through this loose tissue caudad or cephalad to the nearest intervertebral foramen, where they pass outward, piercing the dural sheath. In their course they carry along with them a connective tissue contri-

bution from the pia and dura, which, through the intervertebral foramina, is directly continuous with the peripheral nerve-sheaths; this tissue furnishes the capsule and framework for the spinal ganglia, which are found just external to the foramina and attached to the fibres of the dorsal roots.

The *pia,* in contrast to the two more external membranes, forms, as we may say, an integral part of the structure of the cord, and serves to some extent as a framework, inasmuch as it is closely adherent to the peripheral layer of neuroglia and follows the outline of the cord entering all clefts and depressions. In the anterior median fissure it sinks to the bottom as a thick, strong lamella, *septum ventrale,* supporting, just ventral to the anterior commissure, the *arteria medullaris ventralis.*

The pia throughout is richly supplied with blood-vessels. Branches from these supply the cord, penetrating from the periphery inward and from the arteria med. ventr. outward. The vessels carry with them a connective tissue adventitia derived from the pia. In no case, however, were processes of pia seen entering the substance of the cord except as accompanying blood-vessels. This is easily demonstrated in specimens over-stained with iron hæmatoxylin and differentiated with picro-fuchsin. In such preparations the vessels, together with their connective tissue support, are stained brilliant red in contrast to the yellow-brown neuroglia septa which might otherwise be mistaken for pia.

At three places in its circumference, the pia receives an accession of thick dura like connective tissue fibres, producing ligamentous formations which extend as three longitudinal bands, lens-shaped in cross-section. Two of these are situated laterally, *ligamenta longitudinalia lateralia,* and one is situated at the attachment of the septum ventrale, *ligamentum longitudinale ventrale.* The former corresponds to what *Berger*[6] has described as the ligamentum dentatum in reptiles. Between the 27th and 38th segments in the region of the lumbo-sacral enlargement, these bands reach a special development; they become much stronger and are modified in form. From the ligamentum long. ventr. pointed, tooth-like processes extend laterally to join the ligamenta long. lat. These processes fit closely in the intersegmental grooves, the sulci transversi, of the ventral surface of the cord. This is represented in Fig. 2, a.

A resemblance between this structure and the dural tissue is at once noticed, but it is identified as modified pia from the fact that the arach-

[6] Berger, Ueber ein eigenthümliches Rückenmarksband einigen Reptilien und Amphibien, Sitzb. Wiener Akad. Wiss., Bd. lxxvii, 3 Abth.

noid lies external to it, and separates it from the dura proper. In the intervening spaces the pia becomes thinner and web-like; here the eminentiæ ventrales bulge forward and along their lateral border give off the ventral nerve roots which pierce the pia just ventral to the liga-

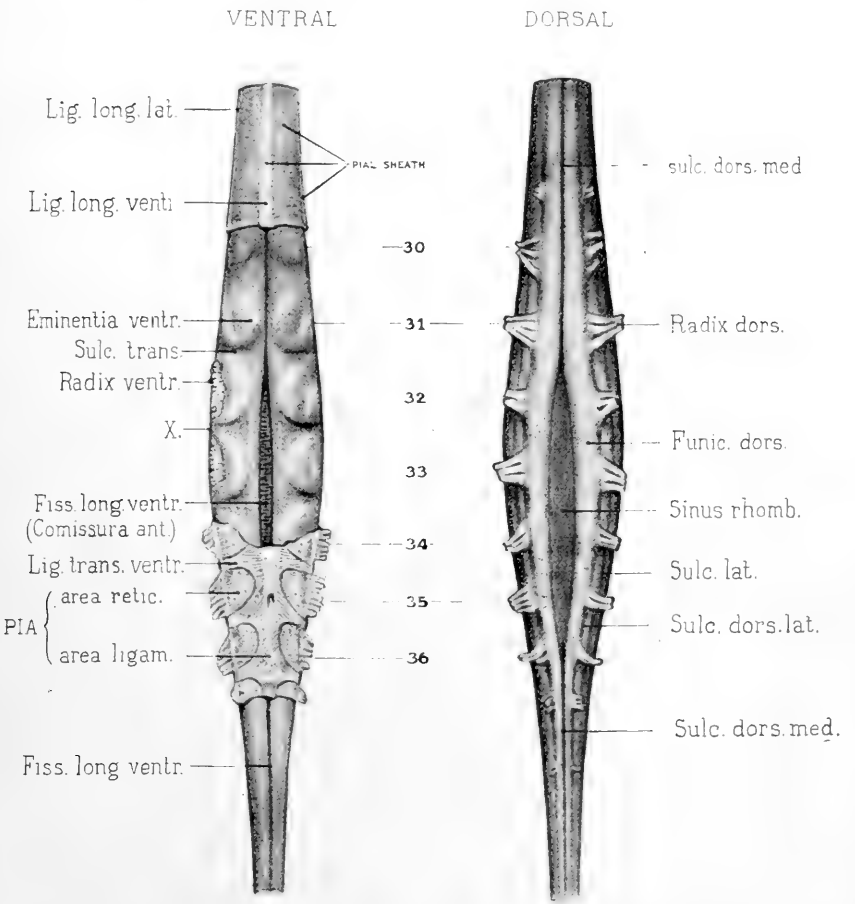

Fig. 2. Ventral and dorsal surfaces of the lumbo-sacral enlargement of the ostrich spinal cord, enlarged to 1⅕ natural size. The pial sheath has in part been stripped off in order to show the eminentiæ ventrales. X indicates the situation of one of the nuclei marginales majores.

menta long. lat. Strong fibrous processes, *ligamenta denticulata,* extend also lateral from the ligamenta long. lat. to the dural sheath and thus render further support to this region of the cord. See Fig. 5.

Caudal to the lumbo-sacral enlargement, the pia returns to the more simple sheath-like form as seen in the thoracic and cervical regions.

A work on the comparative and embryological anatomy of the spinal cord meninges has recently been published by *Sterzi.*[7]

In the mammalian embryo (Ovis aries), 15 mm. long, the author describes a mesenchyma perimeningeale which first produces a definite spinal cord membrane in the embryo of 20 mm. This he calls *meninx primitiva.* In the 80 mm. long embryo this membrane is differentiated into an outer layer or dura mater, and an inner layer or meninx secondaria. The two are separated by an intradural space. The dural layer is separated externally by the epidural space from an *endorhachide* which Sterzi finds always distinct from the dura. In the 157 mm. embryo the *meninx secondaria* is further differentiated into an outer or arachnoideal layer and an inner or pial layer. In his comparative series the author finds the Petromyzon as representing the 20 mm. embryonal stage. The Rana esculenta and Lacerta viridis represent the 80 mm. stage. The development shown by the 157 mm. embryo with a differentiated arachnoid he finds only in the mammals. Our findings in the ostrich do not correspond with this. In Sterzi's series the birds are represented by Gallus domestica in which he describes a meninx secondaria not yet differentiated into pia and arachnoid. In the ostrich we find, as is above described, an arachnoidal layer which presents all the distinguishing features of that of the mammalian cord.

GENERAL MACROSCOPIC DESCRIPTION.

The abrupt change from the slender cervical spinal cord of the ostrich to the thick medulla oblongata gives a rather definite level at which the cephalic end of the cord may be said to be located. From this point extending caudally it stretches throughout the entire length of the spinal canal, its slender tapering end extending to the last coccygeal vertebra. It measures 81 cm. long in a small ostrich, the middle of whose back stands about 60 cm. above ground, and whose head in the ordinary upright position is 45 cm. higher, or 105 cm. above ground.[8]

From each side of the cord throughout its length is given off a series of fine rootlets which unite, within the dural sheath in segmental bundles, to form the dorsal and ventral nerve roots. These, together, pierce the

[7] Sterzi, Anatomia comparata ed all'ontogenesi delle Meningi midollari: Atti del Reale Instituto Venento di Scienze, Lettere ed Arti, Tomo LX, 1900-1901.

[8] All the measurements hereafter stated are taken from this same specimen.

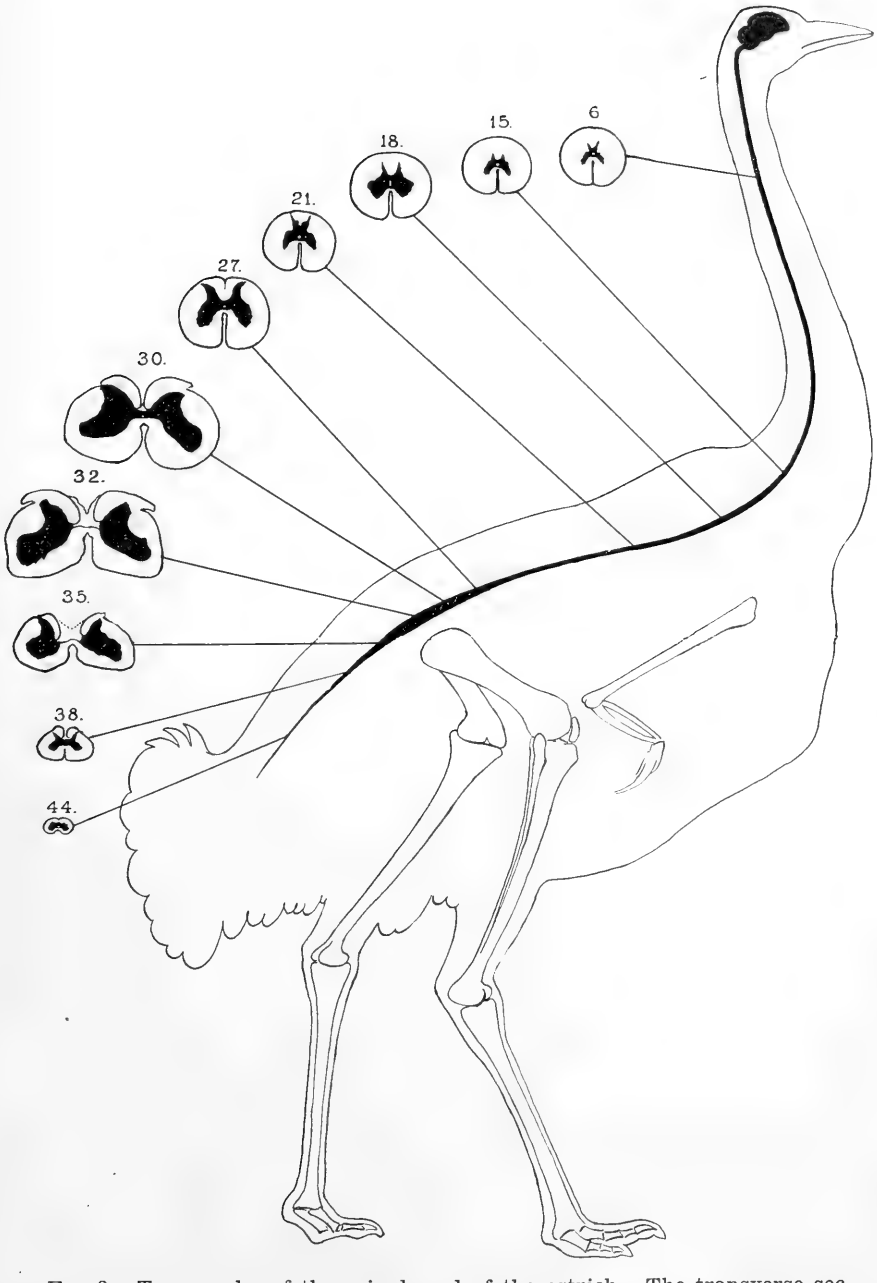

FIG. 3. Topography of the spinal cord of the ostrich. The transverse sections are all made on the same scale of enlargement and their proper levels are indicated on the drawing.

dural sheath, leave the spinal canal, and form the spinal nerves, as is described under the heading Meninges. Owing to the fact that the roots have a short intravertebral course, leaving the canal directly, a bundle of them forming a cauda equina is not here present, and the nerves thus correspond in position to the segments of the cord and to the vertebræ. There are in our specimens 51 pairs of nerves. We may classify the nerves and segments after the morphology of the vertebræ as follows:

Region.	No. of Nerve Pairs.	Segments.
Cervical	15	1st to 15th.
Thoracic	8	16th to 23rd.
Lumbo-sacral	19	24th to 42nd.
Coccygeal	9	43rd to 51st.

The topography of the cord is represented in Fig. 3. It will be observed that corresponding to the wings and legs the cord is in two places increased in size, the *brachial* and *lumbo-sacral enlargements.* The former is so barely visible that one notices at first only the enormously developed lumbo-sacral enlargement. A more careful observation however discloses a slight increase in size in the region lying between the 16th and 19th pairs of nerves. The difference in size is much more apparent in cross-sections.

Fürbringer[9] describes the plexus brachialis of the ostrich as made up of the spinal nerves arising from the 17th to 21st segments, and this corresponds to our cervical enlargement. It is this region, therefore, that we must think of as the sensory and motor center for the wing musculature.

The remainder of the cervical and thoracic portion of the cord is nearly uniform in size, and on section shows a rounded circumference. A *fissura ventralis longitudinalis* is to be seen, but dorsally in this region no fissure is present. The segments in a way are marked off at the attachment of the spinal nerves by a slight dorso-ventral compression and a corresponding increase in size laterally.

In the lumbo-sacral region an entirely different appearance is presented. It is in this region of the cord that the crural and sacral plexuses are attached which supply nerve-fibres to the leg. The system of reflexes which is necessary for the control of the massive musculature of this member demands a large accumulation of nerve-cells and connecting nerve-fibres, and this accumulation forms the lumbo-sacral enlargement, the so-called "Lumbar Brain." As is stated above, the lumbo-sacral

[9] Fürbringer, Untersuchungen zur Morphologie und Systematik der Vögel. Theil I., Amsterdam, 1888.

region of the cord extends from the 24th to the 42nd segment. About one-half of this space, from the 26th to 37th, is occupied by the lumbo-sacral enlargement.

A feature which contributes largely to the peculiar appearance of this part of the cord is the change occurring in the posterior longitudinal sulcus. What was a barely-perceptible furrow in the cervical and thoracic cord becomes, at the beginning of the lumbo-sacral region, more distinct, and, where the 31st pair of nerves are given off, it rather abruptly widens out into a broad boat-shaped groove, the *sinus rhomboideus sacralis*. This reaches ventrally to the commissura anterior, and spreads apart the posterior funiculi from the 31st to 36th segment, at which point the sides again come together and are continued as the posterior longitudinal furrow. This sinus is filled with a delicate gelatinous tissue, the structure of which will be discussed later.

A drawing of the dorsal surface is reproduced in Fig. 2, b; lateral to the sinus can be seen the sharply-defined dorsal funiculi increasing in size from below upward. Each dorsal funiculus is bounded laterally by a dorso-lateral groove, at a point corresponding to the tip of the dorsal horn. Entering this groove are the enormous dorsal nerve roots, grouped into segmental fibre bundles.

Fig. 2, a shows the ventral surface of the enlargement. At two places the pial sheath has been left intact. In this part of the cord the pia is considerably modified from the form which is present in other regions. Beginning at the 26th segment there is a marked increase in the size of the thickened strips of the pial sheath, or ligamentous bands. The pia in the intervening spaces becomes thinner and more web-like. Between the 30th and 37th segments the ligamentum long. ventr. sends out tooth-like intersegmental processes which join the ligamenta long. lat., and the ligamentous structure thus formed affords a strong support where, owing to its specialized character, the cord demands more than ordinary protection.

On removing the pia, there is seen an enlargement of the fissura longitudinalis ventralis, which forms a sinus resembling, to some extent, the sinus rhomboideus of the dorsal surface, though it is shorter and narrower. Moreover it is not filled with the gelatinous semi-transparent tissue as seen in that sinus, and at the bottom one can see the cross-fibres of the commissura anterior. The space where the fissura long. ventr. may be called a sinus, extends from the 31st to the 35th segment, and is 1.3 mm. wide.

The great increase in the anterior horn elements, which occurs in the enlargement, is segmental in character, and forms segmentally projecting

masses of grey substance whose outline can be seen on the ventral surface of the cord as rounded elevations, *eminentiae ventrales,* which bulge forward through the ligamentous framework. There is thus formed a series of hill-like prominences separated by intersegmental grooves, *sulci transversi.* In the grooves lie the lateral prongs of the ligamentum long. ventr. From the lateral border of the segmental elevations arise the motor nerve roots as a row of fine rootlets which pass through the web part of the pial sheath just ventral to the ligamenta long. lat.

On examining the lateral surface of the cord in the region from the 31st to 36th segment, one sees, just dorsal to the ligamenta long. lat., at the level of each sulcus transversus, a small oval greyish projection measuring 1.4 mm. long and 0.4 mm. wide. These projections are the *nuclei marginales majores,* or the Large Hofmann Nuclei. They are easily seen with the naked eye, but better with a lens and under water. A description of them will be included under the heading Nerve-Cell Groups.

Caudal to the lumbo-sacral enlargement the cord decreases abruptly in size and extends, gradually tapering, to the end of the spinal canal. There is no cauda equina. A section of the most caudal pieces of our specimen shows a central canal and a similar general arrangement of grey and white matter as present in other parts of the cord.

From *Gadow's*[10] work we can localize the peripheral parts that are controlled by this region of the cord. Gadow describes the sacral plexus as consisting of three individual groups: plexus cruralis; plexus ischiadicus; plexus pudendus. The nervus sacralis, which, by means of its bifurcated root, joins the latter two plexuses, he locates in the ostrich at the 37th segment. The nervus furcalis, which separates the plexus ischiadicus from the plexus cruralis, he places at the 31st segment. Thus the plexus cruralis is attached to the cord from the 27th to the 31st segment, or the cephalic half of the enlargement. We may, therefore, locate here the nerve-cell groups belonging to the trochanter muscles and the muscles situated on the medial and anterior side of the femur, to which area the plexus cruralis is distributed. The plexus ischiadicus arises by 7 roots from the caudal half of the enlargement, the 31st to 37th segment. The roots of this plexus unite to form nervus ischiadicus which supplies the massive group of muscles on the lateral and posterior sides of the femur and the muscles of the lower leg. Caudal to the 37th segment is situated the pudendal plexus which innervates the anal and genital musculature. Beyond the 43rd segment arise the delicate caudal nerves which supply the coccygeal muscles.

[10] L. c., p. 406

Arrangement of White and Grey Substance.

A cross-section of the cord shows, in a general way, a central four-horned area of grey matter surrounded by a much larger area of white matter. The two dorsal horns of grey matter separate off a portion of the latter forming the dorsal funiculi, so called in distinction to the remainder of the white matter, or ventro-lateral funiculi. The outline and relative size of these individual areas in different levels of the cord are shown in Fig. 3.

A great variation exists in the size of the dorsal and ventral horns, as well as the anterior commissure. These structures are apparently closely interrelated, as they undergo the size-variation in unison. All of them reach their greatest development in the lumbo-sacral enlargement. Of the ventral and dorsal horns, the latter show less increase in size in the two enlargements. In the cervical region the dorsal horns are reduced to a narrow strand of grey matter and fail to reach the border of the cord. The white commissure, commissura ventralis, connecting the two halves of the cord is present at all levels, and will be described more in detail in connection with the fibre tracts. The grey commissure from the 31st to the 36th segments entirely fails. Its place is filled by the tissue of the sinus rhomboideus.

A more exact knowledge of the total area of transverse sections made at different levels, and the relative area of the antero-lateral funiculi, the dorsal funiculi, and the grey substance was obtained by a method which allows the calculation of the areas in square mms.

In this method one makes a series of outline drawings (in our case the Edinger drawing apparatus was used) of the various segments on a sheet of evenly-rolled lead or tin foil. Thick cardboard can also be used when the drawings are large. The drawings of the individual segments thus outlined on the sheet of lead are all magnified on the same scale. A drawing is also made in a similar way and with the same enlargement of a square cm. which has been outlined in ink on a glass slide. The drawings of the different segments and of the square cm. are then cut out from the lead sheet, and the segments further cut apart into the different areas. These pieces are all separately weighed. The ratio then, between the weight of each individual part and the weight of the piece representing the square cm., is equivalent to the area of this part.

Sections were taken from each segment of the ostrich cord, and the area of the various fields was thus calculated. The sections were taken uniformly near the departure of the nerve to avoid the discrepancy that might occur from differences in the same segment. This variation in the upper part of the cord is hardly appreciable. In the lumbo-sacral enlargement, however, it is more marked, and we have a distinct segmental character given to the cord by the increase in the size of the ventral horns, which occurs in the middle of the segment. Taking the sections at the level of the roots has the

further advantage that here the boundary of the dorsal funiculi is more sharply defined, owing to the larger number of entering dorsal root-fibres.

The results of the method in our case are represented in the adjoining table:

TABLE

SHOWING SIZE OF VARIOUS AREAS OF CROSS-SECTIONS OF CORD AT DIFFERENT LEVELS. THE NUMBERS GIVEN INDICATE SQUARE MMS.

Segment.	Grey Matter.	Ventro-lateral Funiculi.	Dorsal Funiculi.	Total Area.
3	.7	8.7	.7	10.1
5	.7	9.1	.7	10.5
7	.7	9.1	.7	10.5
12	.8	9.2	.7	10.7
13	.9	8.8	.7	10.4
14	.9	8.9	.7	10.5
16	1.3	10.9	.9	13.1
17	1.9	12.2	1.1	15.2
19	1.7	10.9	.9	13.5
20	1.3	9.9	.7	11.9
21	1.2	9.9	.6	11.7
22	1.2	8.9	.5	10.6
24	1.5	10.5	.7	12.7
26	2.1	11.9	1.1	15.1
27	4.4	14.9	2.1	21.4
28	6.3	18.3	3.1	27.7
29	8.6	21.9	4.3	34.8
30	9.6	23.5	5.4	38.5
31	7.8	18.9	4.4	31.1
32	7.2	16.6	3.5	27.3
33	5.9	14.2	3.1	23.2
34	4.8	9.3	2.1	16.2
35	3.5	6 9	1.1	11.5
36	1.9	5.2	.6	7.7
38	1.0	3.1	.5	4.6
44	.4	.9	.2	1.5

These areas and their relative size are more graphically represented in the diagram given in Fig. 4. The size of the grey substance, the ventro-lateral funiculi, and the dorsal funiculi in typical segments are represented by curves, the height of which signifies square mms. as shown by a scale on the left.

From this diagram it is apparent that the ventro-lateral funiculi form by far the greatest area at all levels. The proportion is much greater above than below the lumbo-sacral enlargement. This could be accounted for in part by the presence of tracts connecting the enlargement with the brain centers. In both the cervical and lumbo-sacral enlargements the increase in area of the ventro-lateral funiculi is greater than that of the grey matter and dorsal funiculi. This is doubtless due to the large number of association fibres which form a field of fine fibres surrounding the anterior horns.

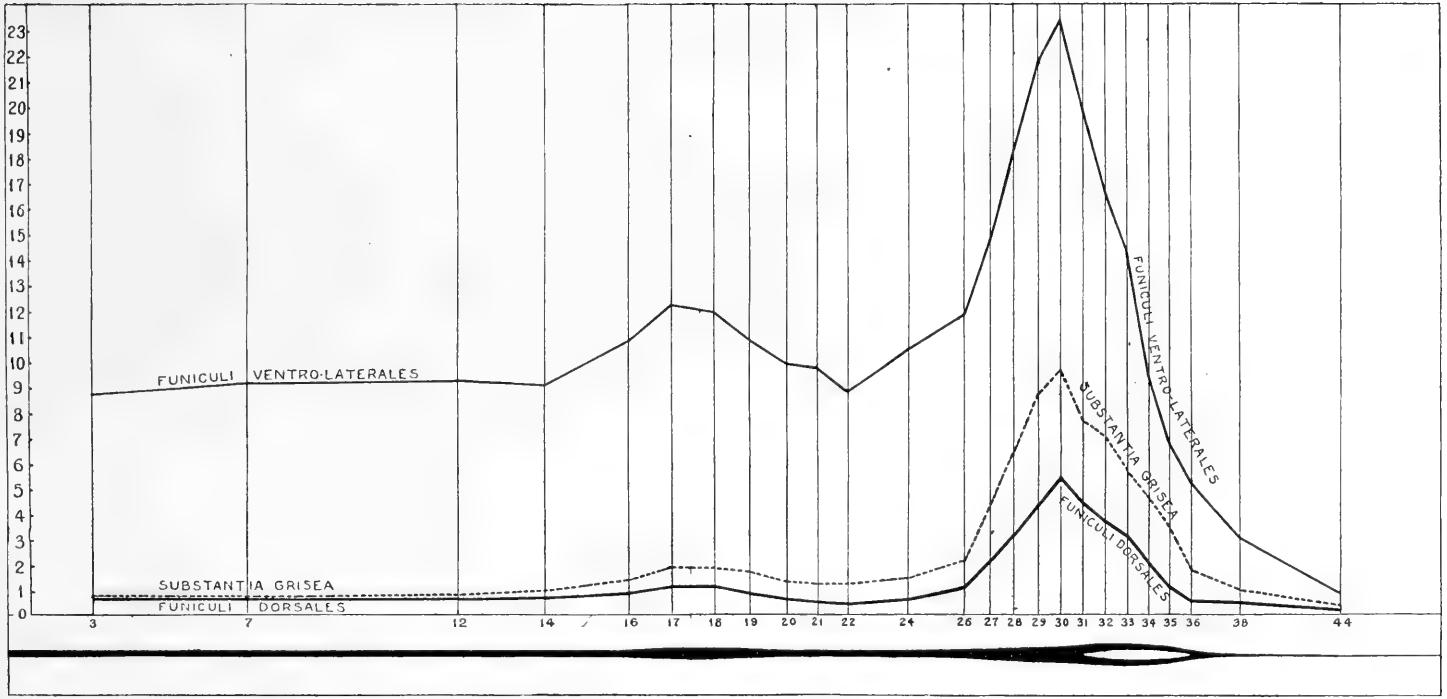

FIG. 4. Diagram showing the size of the various areas of the cord at different levels. The curves are drawn on a scale which shows the size in square mms. The spinal cord is drawn in below the diagram and represents the general size for the corresponding segments.

Between the curves which represent the grey matter and the dorsal funiculi there is a closer uniformity in size; although the former shows a greater increase in the regions corresponding to the wing and leg musculature.

Of all three curves on the diagram that of the dorsal funiculi indicates the smallest as well as the least variable area. It is smallest at the 44th segment, and presents practically no change as we proceed cephalad until the 36th segment. If we look at Fig. 2, b it is to be seen that the dorsal nerve roots from the 36th to 31st segment are enormously increased in size. Corresponding to the entrance of these large dorsal nerve roots, in the same segments in the diagram there is an abrupt ascent of the dorsal funiculi curve. Attention is called to the fact that the increase in the size of the dorsal funiculi extends cephalad from the point of increased dorsal root fibres. Therefore we may assume that the collaterals in the dorsal funiculi extending caudalward from the dorsal roots are either very few in number or very small in diameter, and that the general course of the entering impulses is in the cephalic direction.

The descent of the curve of the dorsal funiculi from the 30th to the 26th segment is as abrupt as the previous ascent. While in a space of six segments the area of the dorsal funiculi was increased nine times in size, this area, four segments higher up, has lost already more than three-fourths of this increase, and so the area at the 26th segment is only one-fourth of that at the 30th. If we take for granted that all the fibres that leave the dorsal funiculi enter the grey substance. and that there is very little variation in the size of the fibres from the 30th to 26th segment (both of which facts are confirmed by microscopical study of the cross-sections) then we may say that three-fourths of the fibres present in the dorsal funiculi at the 30th segment have entered the grey substance before the 26th segment. In other words *the course of the dorsal root fibres within the dorsal funiculi is a short one, and not more than a small proportion of these fibres ever reach the medulla by this tract.*

That which is apparent regarding the dorsal funiculi in the lumbosacral enlargement is seen again in the cervical enlargement, though in the latter it is less marked. Above the cervical enlargement the rate of accession and loss of fibres in the dorsal funiculi maintains a constant balance, and the curve of area runs as a horizontal line.

FINER STRUCTURE OF THE CORD.

By the usual methods of staining, the cord resolves itself into three elements: Neuroglia, which forms the general framework; Nerve-Cells

and Myelinated Axis-Cylinders, which form the fibre tracts and make up the bulk of the white substance. The histology of the cord will be discussed under these heads.

The *neuroglia* was studied in preparations stained by the iron hæmatoxylin picro-fuchsin method of Weigert. This method cannot be spoken of as a glial stain; on the contrary, the glia does not stain with fuchsin as in the original Van Gieson method, but remains a yellowish brown and is seen in sharp contrast to the brilliant red connective-tissue elements. By combining the original Van Gieson method and the Weigert modification we may study the glial distribution by a process of exclusion; this permits the following general description:

The glia fibres are more numerous in the grey substance than in the white, and are more numerous in the ventral horns than in the dorsal horns. They form an especially thick mass in the region of the central canal. In the white matter on the periphery, adjoining the pia, is a rather uniform layer of closely-lying fibres which forms a glial sheath to the cord, the *peripheral glia sheath.* This layer, at a point corresponding to Lissauer's fasciculus, is thickened and extends into the substance of the cord as a broad strand to meet the tip of the dorsal horn, which fails to reach the border of the cord. This strand spreads laterally to the dorsal horn and forms the web-like *formatio reticularis* situated in the median part of the lateral funiculus.

In most sections another glial process is seen extending from the sulcus longitudinalis dorsalis toward the central canal, the *septum longitudinale dorsale,* supporting a blood-vessel with its connective tissue sheath. Aside from the peripheral sheath and the processes as mentioned, the glia of the white substance forms a more or less uniform framework, supporting the nervous elements proper. There remains to be mentioned a special modification of the glial arrangement associated with the formation of the sinus rhomboideus.

Sinus Rhomboideus.

A macroscopic description of this structure has already been given, and we have spoken of the delicate gelatinous tissue with which it is filled. From the study of a series of transverse sections through this region it is our conclusion that this tissue is not a new structure, but is identical with the peripheral glia sheath and the septum dorsale which have become modified in their histological character.

In sections through the 29th segment there is a marked increase in the size of the sulcus longitudinalis dorsalis, which penetrates ventrally one-half the length of the septum dorsale and splits it in wedge-shape

fashion. In the 30th segment the sulcus extends the entire distance to the grey commissure completely separating the dorsal funiculi and forming the cephalic end of the sinus rhomboideus. At this level a change in the character of the glia shows itself in that part of the peripheral sheath between the ventral and dorsal nerve roots, as well as in the grey commissure and the adjoining divided septum dorsale. In these places instead of a compact mass of fibres the glia shows a looser and more sponge-like appearance. In the succeeding sections this glial modification rapidly increases in extent, coincident with the increase in the size of the sinus, and reaches its maximal development between the 30th and 36th segments. A drawing from this region is reproduced in Fig. 5, and a portion of the glial web is shown under higher magnification. It is thus seen that the peripheral glia sheath throughout the circumference of the cord, except at the attachment of the ligamenta denticulata, is changed into, or replaced by, a tissue consisting of enormous cells (.003 to .004 mm. in diameter), the body of each of which is filled with a transparent fluid of undetermined nature which crowds the small nucleus to one side, or the nucleus is suspended in the fluid supported by a slender stalk of cell tissue. It resembles fat tissue to some extent. It however fails in frozen section to stain with Herxheimer's solution of *Fettponceau*. In iron-hæmatoxylin picro-fuchsin preparations there is no trace seen of connective tissue fibres. The cells remained unstained like the neuroglia cells of other parts of the section. By exclusion, then, we are led to consider them as modified neuroglia cells, though we unfortunately lack the definite evidence of a selective stain.

The sinus rhomboideus of birds has always been an object of interest to investigators, especially as to the character and significance of the gelatinous material with which it is filled. Of the earlier writers the work of *Duval*[11] may be referred to, the results of which were more or less confirmed recently by *Kölliker*.[12] Both of these authors from embryological evidence agree as to the glial nature of the tissue filling the sinus. They, however, do not make mention of the presence of this web-like material around almost the entire circumference of the cord.

The grey commissure and the septum dorsale are entirely changed into this tissue, which thus fills the sinus as a broad network separating the blunt ends of grey substance and the dorsal funiculi and extending ventralward to the commissura ventralis. In the ventral part lies the

[11] Duval, L. c.

[12] Kölliker, Ueber die oberflächlichen Nervenkerne im Marke der Vögel und Reptilien. Zeitschrift f. wiss. Zool., LXXII, 1.

central canal held in suspension by a few coarser strands of glia fibres which lie among the cells and bridge over the space separating the grey matter. This meshwork formation of these modified glia cells extends somewhat into the territory of the white substance along the borders of the dorsal, lateral and ventral funiculi. Under low power this ragged edge of white substance gives the deceptive appearance of an artifact.

From the 38th segment caudalward there is a gradual retrogression of neuroglia to the form as previously described.

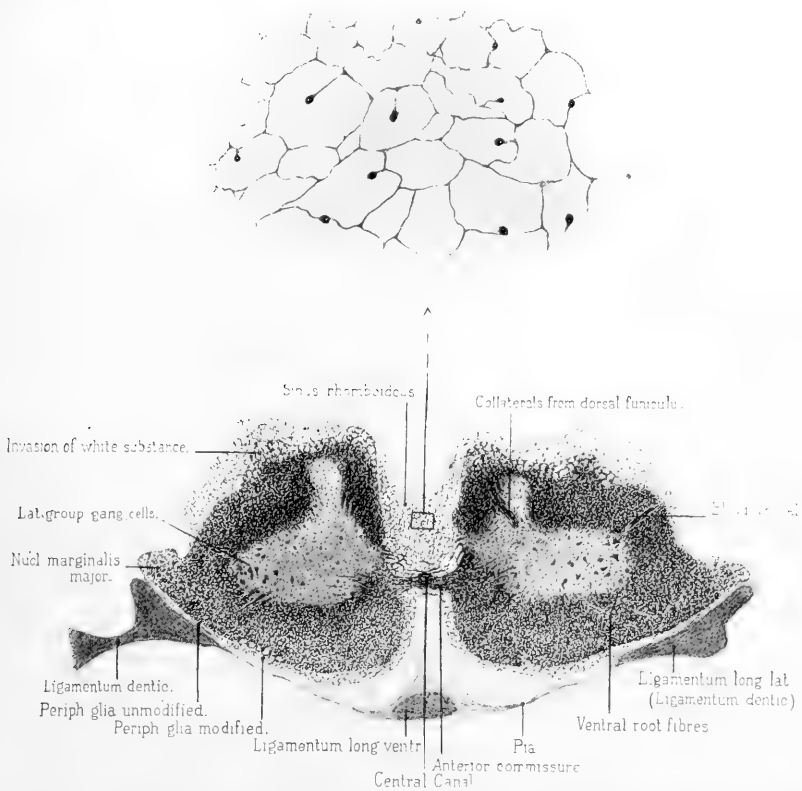

Fig. 5. Cross-section of the lumbo-sacral enlargement of the ostrich spinal cord, at the 36th segment, enlarged 12×. A portion of the sinus rhomboideus tissue is shown above with an enlargement of 270 ×.

Central Canal.

The cylindrical epithelial cells lining the central canal form a layer .007 to .015 mm. thick which is supported by a thick mass of glial tissue,

the *substantia gelatinosa centralis,* in the middle of the grey commissure. Where the grey commissure is lacking, in the region of the sinus rhomboideus, the central canal is supported just dorsal to the commissura ventralis by the loose strands of glial fibres which bridge over the space between the blunt ends of grey substance.

The lumen of the canal varies in irregular manner from round to oval, and where it lacks the support of the grey commissure it is no more than a narrow slit. Where it is round or oval it has a diameter averaging from .035 to .04 mm. In both cross and longitudinal stained preparations there is seen within the lumen the so-called *Reissner'sche Centralfaden. Kölliker*[13] in a recent contribution gives the opinion that it is a "natürliche Bildung beim Vögel, Reptilien, und Amphibia," and also finds in it "eine überraschende Aehnlichkeit mit einem Achsencylinder." This is contrary to *Gadow*[14] who considers it a product of shrunken cerebro-spinal fluid and lymph corpuscles. In favor of the view as held by Gadow may be stated the three following facts: The structure shows a marked and irregular variation in form and size in different sections; in some transverse sections it was seen as multiple "Centralfäden"; in sections stained with toluidin blue it retains a deep blue stain while the axis-cylinders in all other parts of the section are unstained.

Nerve-Cell Groups.

The majority of the nerve-cells of the spinal cord of the ostrich are situated in the grey matter of the ventral horn. There are, however, many cells in the grey commissure and the dorsal horn, and there are still other cells among the fibres of the white substance, especially near the periphery. These cells vary at different levels in their form, size, and manner of grouping. For their descriptions the following classification has been found advantageous:

1. Lateral Group—	a. Lateral cells.
	b. Dorso-lateral cells.
	c. Ventro-lateral cells.
2. Central Group—	a. Small mixed cells.
	b. Giant cells.
3. Commissural Group—	
4. Dorsal Group—	a. Clarke cells.
	b. Dorsal horn-cells.
5. Peripheral Group—	a. Nuclei marginales majores.
	b. Nuclei marginales minores.
	c. Scattered cells.

[13] Kölliker, L. c., p. 159.
[14] Gadow, L. c., p. 338.

The *lateral group* consists of more or less uniformly large multipolar cells, which in finer histology closely resemble the motor cells of the ventral horns of the higher vertebrates. Their distribution in typical sections is shown in Fig. 6. They are seen in every section, but vary in number, being most numerous in the lumbo-sacral region and least numerous in the cervical segments. Corresponding with the number there is some variation in the size; those in the cervical segments average .02 mm. in diameter, while in the lumbo-sacral region there are many cells over .04 mm. This group may be further subdivided into cells having lateral, dorso-lateral, and ventro-lateral positions. A particularly well-defined group of the ventro-lateral cells occurs in the region of the sinus rhomboideus (Fig. 6, segm. XXXVI).

If we compare this lateral group with the cells of the human cord as classified by *Waldeyer,*[15] it is apparent that it corresponds to his median and lateral groups, each of which he subdivides into anterior, middle and posterior subgroups. The cells of the lateral group in segment XXXVI could have been separated in a similar manner into a median and a lateral group; the ventro-lateral cells would then well correspond to Waldeyer's median group, and the lateral group could be further subdivided into anterior, middle and posterior groups. Such a classification in the ostrich however serves only irregularly and for isolated segments, and therefore this distinction between the cell groups was not attempted; but all the large multipolar cells of the ventral horns, the so-called motor cells, were put under the one general class, the lateral group, as described above.

Most of the cells of the lateral group apparently send their axis-cylinders into the ventral nerve-roots. The axis-cylinders of the ventro-lateral cells, however, seem to enter the commissura ventralis. No attempt to establish such relations could be made without Golgi preparations, and these unfortunately were not to be had from our material.

The *central group* occupies the area of junction of the ventral and dorsal horns, and invades the territory of the horns proper. It consists of loosely-scattered cells which vary greatly in size and average a third smaller than the cells of the lateral group. They also stain less intensely and have fewer processes, consequently having less tendency to a multipolar form.

In the lumbo-sacral enlargement there appear cells among this group which from their size we may speak of as *giant cells.* They are quadrilateral or rounded in shape, and vary from .03 to .09 mm. in diameter.

[15] Waldeyer, Das Gorillarückenmark, Abhandl. der kgl. preuss. Akad. der Wissensch. zu Berlin, von Jahre 1888.

They are distinguished from the Clarke cells and cells of the lateral group by having fewer processes, by their tendency to easy disintegration, staining less intensely and having finer granules in the cell body. These cells are present throughout the whole enlargement, but are more numerous in the upper part (27th to 31st segments). A

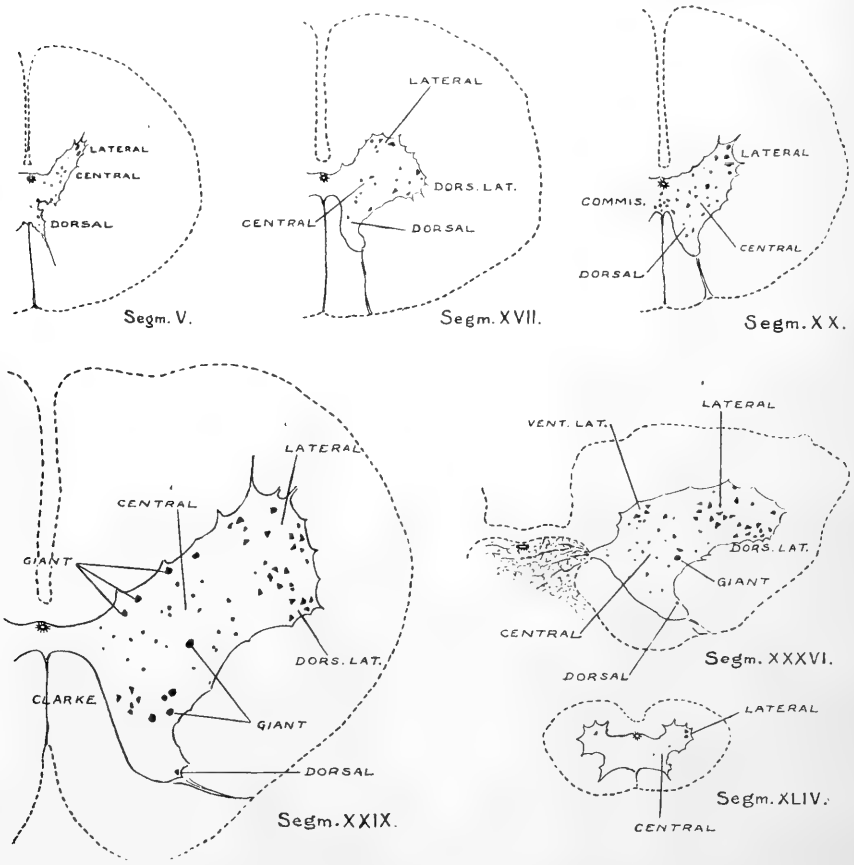

Fig. 6. Cell-groups in the grey substance of the spinal cord of the ostrich.

few are also seen in the 13th to 16th segments, just above the cervical enlargement. As can be seen in Fig. 6, segm. XXIX, they are scattered over the entire area of the central group. Very often they are seen on the extreme ventral or dorsal border of the grey matter. The largest number seen in any one section (20 μ thick) was eight. These giant cells present a striking similarity to the large cells seen in the lateral group of the nucleus funiculi gracilis of the human medulla.

The *commissural group* is made up of a compact group of small intensely-staining multipolar cells, which are found in the grey commissure in the thoracic division of the cord, from the 20th to 27th segments. It suggests, by its position, a possible relation with the viscera.

The *dorsal group* includes in sections through the 26th to 31st segments a small group of cells on the median border of the grey matter at the junction of the two dorsal horns. The cells of this group resemble those of the lateral group, though slightly smaller. From their similarity, in position and appearance, to the group in the mammalian cord these are classed as Clarke cells (see Fig. 6, segm. XXIX). Otherwise as noteworthy are classed under the dorsal group the occasional small multipolar or spindle-shaped cells, which are seen on the periphery of the dorsal horn both median and lateral, and frequently on the tip of the horn near the entrance of the dorsal root.

Peripheral Group.

In 1889 *Lachi*[16] described a peripheral group of nerve-cells forming a series of segmental projecting nuclei, occurring in the lumbo-sacral enlargement of the spinal cord of doves. This nucleus was seen later by *Gaskell* and *Schafer* but attracted little attention until *Kölliker*,[17] originally unaware of Lachi's work, published the results of a most complete study of this structure, both in the embryo and the adult bird. Kölliker finds three varieties of peripheral cell-groups, namely: Hofmann'sche Grosskerne, so named after his Präparator P. Hofmann, who had called his attention to them; Hofmann'sche Kleinkerne; and a scattered group.

In the embryonal cord, 4½- to 5-day chick, Kölliker describes a group of cells separating itself from the superficial cells of the ventral horn. This group in the 10-day chick is completely separated and forms a definite peripheral nucleus, the Hofmann'sche Kerne. There are 28 of these nuclei on each side of the cord, segmentally arranged according to the 28 spinal nerves and ganglia. Of these nuclei the 5 or 6 pairs, corresponding to the level of the sinus rhomboideus, undergo a marked development, and in the 15-day chick can be seen bulging from the periphery of the cord just dorsal to the ventral nerve roots, Hofmann'sche Grosskerne. The nuclei in the other regions of the cord do not share this development, but remain more or less

[16] P. Lachi, Alcune particolarità anatomische del ringonfiamento sacrale nel midollo degli uccelli, Memorie della Società Toscana di Scienze Naturali. Vol. X, Pisa, 1889.

[17] Kölliker, a.—Ueber einen noch unbekannten Nervenzellenkern in Rückenmark der Vögel, Akad. Anzeiger (Wien), Nr. XXV, 1901. b.—Weitere Beobachtungen über die Hofmann'schen Kerne am Mark der Vögel, Anatom. Anzeiger, Bd. XXI, Nr. 3, 1902. c.—L. c.

rudimentary, the Hofmann'sche Kleinkerne. The third or scattered group is made up of cells similar to the lateral group cells of the ventral horns, and occurring at irregular points on the periphery of the ventro-lateral funiculi, more especially near the exit of the ventral nerve roots and near the Hofmann'sche Grosskerne. Kölliker considers these cells to be detached elements from the ventral horns.

In the ostrich the occurrence and arrangement of peripheral cells is similar to that found by Kölliker and Lachi in the dove and hen. In describing them we follow Kölliker's classification, but would substitute more descriptive names.

The *nuclei marginales majores* (Lobi accessori.—Lachi; Hofmann'sche Grosskerne.—Kölliker) lie on each side of the cord just dorsal to the ligamenta longitudinalia lateralia at levels marked off by the sulci transversi ventrales. Of these nuclei 6 pairs could be readily seen with the naked eye. They appear as elongated oval greyish semi-translucent elevations measuring macroscopically 1.0 to 1.4 mm. long. The interval between successive nuclei averages 6.0 mm. Each segment of the lumbo-sacral region was cut in transverse or longitudinal series, mostly the former. In studying these sections this nucleus was identified in the 30th, 31st (32d injured in removing the cord), 33d, 34th, 35th, and 36th segments. Thus the nucleus occurs in the region of the sinus rhomboideus, extending a little cephalad as well as somewhat caudad to it. Microscopically in the preparations the nucleus is seen projecting from the lateral border of the cord just dorsal to the attachment of the ligamentum denticulatum, as is represented in Fig. 5.

The size of the nuclei averages among the larger ones .10 to .18 mm. antero-ventral diameter, and .08 to .12 mm. lateral diameter. In a continuous series of sections 20 μ thick the nucleus is present in 62; that is the nucleus is 1.24 mm. long. These dimensions are somewhat smaller than the macroscopic, as could be expected from shrinkage associated with the embedding process, and possibly partly due to greater accuracy in measuring, the boundaries of the nuclei being more definite in the prepared and stained specimen.

The free border of the nucleus is overlapped by pia, and the inner border merges gradually into the white substance of the cord. It consists of a network of glia tissue, somewhat looser and more vascular than the adjoining cord. In this sponge-like framework lie a number of multipolar nerve-cells and myelinated axis-cylinders. The cells resemble those forming the lateral group of the ventral horn, but are not more than one-fourth to one-sixth as large. In one nucleus 10 of these were seen in which the cell nucleus was cut through. In the

majority of sections there are not more than 5 such cells present. The myelinated axis-cylinders have mostly a longitudinal course, and are about the same size as those in the neighboring periphery of the cord. Throughout the greater part of the nucleus they are uniform in number, 112 were counted in one section, but such a count is subject to error as it is often difficult to say whether the fibres belong to the lateral funiculus or to the nucleus owing to the indistinct inner border of the latter. In studying a complete series of transverse sections through the nucleus, prepared after Weigert's myelin-sheath method, one gets the impression that these large axis-cylinders belong properly to the lateral funiculus. In such sections near its caudal and cephalic ends the nucleus appears as a small island of increased glia tissue lying in the midst of the axis-cylinders near the border of the cord. In the succeeding sections this glia tissue rapidly increases in amount and envelops and carries with it the surrounding nerve-fibres, until finally it bulges from the side of the cord as an exuberant overgrowth. The large size of the nerve-fibres compared to the cells of the nucleus, and their uniformity in number at different levels, would also lend support to the view that they are independent of the cells and not properly a part of the nucleus. There are, however, a certain number of fine axis-cylinders seen in the sections, both with longitudinal and oblique course, which may be related to the cells embedded in the nucleus.

The *nuclei marginales minores* (Hofmann'sche Kleinkerne) are seen in sections taken from the cervical region at levels where the nerve roots make their exit from the dural sheath and vertebral canal. Their size and general position are indicated in Fig. 1. They do not project from the periphery of the cord and have no appearance of activity. The cells are small and are not definitely multipolar. The glia in which they lie is only slightly increased over that present in other regions of the periphery of the cord.

The *scattered group* includes multipolar cells similar to those of the ventral horn, both in shape and size. One or two of these are found in nearly all sections of the lumbo-sacral enlargement, lying among the fibres of the periphery of the ventro-lateral funiculi. They are found most often near the nuclei marginales majores, or among the fibres leaving the cord as the ventral root. It is this group that Kölliker regards as detached elements from the ventral horns.

In regard to *Kölliker's*[18] suggestion of a relation between the Hofmann'sche Grosskerne and the enormous size of the commissura ven-

[18] Kölliker, L. c. (c.), p. 176.

tralis we may state the fact that a longitudinal ventro-dorsal section cut through the commissura ventralis, from the 32nd to 34th segment, shows that the commissure here is practically uniform in the ventro-dorsal diameter. It presents no segmental increase in size at the levels of the Hofmann'sche Kerne which would be expected if the size of the commissure in the lumbo-sacral enlargement were due to the presence of these nuclei.

Fibre Tracts.

Myelinated fibres are present both in the grey and white substance of the cord. In the former they are seen in the preparations in cross and longitudinal section, and form a network which cannot be resolved into definite fibre tracts.

The great bulk of the spinal cord fibres make up the white matter, and form a thick envelope surrounding the grey substance. This envelope may be separated into ventral, lateral and dorsal funiculi. The boundary between the first two is an artificial one, produced by the fibres of origin of the ventral nerve roots. At levels where those fibres are few or absent there is no point of division between the two funiculi.

The *dorsal funiculi* are more sharply defined. They are separated from each other by the septum posterior, and separated from the lateral funiculi by the dorsal horns and the glial processes which extend from the tip of the horns to the peripheral sheath of the cord.

The general variation in size and shape of the dorsal funiculi occurring at different levels of the cord can be seen in Fig. 3. The definite area is recorded by a table and by Fig. 4, in which a diagram gives the area in a curve indicating square mms. Thus a further mention of the shape and size of these funiculi is here not necessary.

In their finer structure the dorsal funiculi consist of fibres of entrance and departure, and fibres having a longitudinal course. The bundles of fibres entering as the dorsal nerve roots vary greatly in size, as is seen macroscopically. Those in the lumbo-sacral enlargement are two or three times larger than those in the cervical enlargement, and about five times larger than those of the upper cervical region. These fibres enter obliquely as a compact bundle at the dorso-lateral border of the funiculi. The bundle then breaks up into loose strands, disappearing among the longitudinal fibres. No fibres could be seen to enter the grey matter directly. In longitudinal sections most of the fibres could be seen to bend upwards, and could be traced a short distance in the longitudinal direction. A few fibres were seen which, on entering, turned caudalwards. In neither Van Gieson nor Weigert preparations, how-

ever, was a "Y" form seen, where the entering fibre had both a cephalad and caudad collateral. That so few fibres take a downward course accounts for the fact that in the 35th and 36th segments, where there is a pronounced increase in the size of the dorsal nerve roots, the corresponding increase in the size of the dorsal funiculi is in the cephalic direction. Furthermore, the course of these fibres in the dorsal funiculi cannot be a long one, and this is shown by the rapid decrease in the size of the funiculi coincident with the decrease in the number of entering fibres, a fact which we have already referred to in the consideration of the diagram, Fig. 4.

The collaterals from the dorsal funiculi to the grey matter vary in number in correspondence to the number of fibres from the dorsal nerve roots. In the lumbo-sacral enlargement these fibres enter the dorsal horn as a large strand of fibres which could be traced to the region at the base of the horn. In the cervical region fibres entering the grey matter are found only as single separate collaterals.

No definite subdivision of these funiculi into separate fasciculi or tracts could be made. In general, however, the fibres of the ventral one-third are smaller and form a triangular field of fine fibres, averaging .2 μ. These are apparently association fibres. This field is not present in the lumbo-sacral enlargement; here the grey commissure is absent, and the dorsal funiculi are separated by the sinus rhomboideus and lie further dorsal. The size of the fibres of this enlargement is uniformly large, the myeline ring in Weigert preparations averaging 1.0 to 1.5 μ. We have already seen that the majority of the fibres of this region do not remain in the dorsal funiculi for a course of more than 3 to 4 segments, and that a small proportion of them reach the medulla through this tract. *It would seem, then, that large fibres do not necessarily indicate long fibres;* because in the lumbo-sacral enlargement the fibres are uniformly large and it is right here that we have shown that at least three-fourths of the fibres have a course in the dorsal funiculi shorter than 4 segments.

The *lateral funiculi* present an inner zone of fine fibres and an outer zone of coarser fibres, the latter fibres averaging 1.0 μ. The inner zone, or formatio reticularis, makes up a third to one-half the area. It is connected with the grey substance by numerous radiating strands of fibres, and apparently consists of association bundles. The outer field is connected with the central grey substance by less numerous strands of fibres. It is in this outer zone that *Friedländer*[19] found ascending

[19] Friedländer, L. c.

and descending cerebellar tracts by experimental secondary degeneration in doves.

The *ventral funiculi* have an inner zone which is a ventral extension of the inner zone of the lateral funiculi. The outer zone, tractus cerebello-spinalis ventralis medialis of Friedländer, is somewhat larger, and forms a more or less triangular field, of which the fissura ventralis forms one side. The fibres of this field are all large and average 1.5 μ, many of them being over 2 μ. In the lumbo-sacral enlargement the enormous increase in size of the ventral and lateral funiculi seems due to an accession of smaller fibres which are added to the inner zone, and this increase is more marked in the ventral than in the lateral funiculus.

A *commissura alba anterior* of obliquely crossing fibres is present at all levels of the cord, connecting the two ventral funiculi. It is greatly increased in size between the 28th and 36th segments. A sagittal section through the commissure in this region does not show any segmental grouping of these fibres. In Weigert preparations strands of fibres can be traced through the commissure coming from the outer zone of the ventral funiculus and extending to the opposite ventral horn. We have here doubtless a motor tract from higher centers, the fibres of which decussate before ending about the cells of origin of the motor nerve roots. The large number of ventral horn-cells in the lumbo-sacral enlargement would thus partly explain the large size of the commissure which here prevails. No trace of commissural fibres dorsal to the grey commissure was found in any of our sections. A posterior white commissure is apparently lacking.

RÉSUMÉ.

In looking back at the more important characteristics presented by the spinal cord of the ostrich, a feature to be first referred to is that in its mass the cord forms by far the largest part of the central nervous system. In other words, then, we have here an animal the various parts of whose body receive their principal innervation from the spinal cord, and the influence of the brain on these parts is secondary and remote—*an animal that works chiefly with its primary apparatus.*

This suggestion as to the important part played by the primary nervous complex is further confirmed by the fact that the grey substance and associating collaterals *vary in amount at different levels according to the demands made by the parts supplied.* Thus throughout the cervical cord where there is a small and uniform number of neck muscles to be supplied the primary apparatus presents a correspondingly small

and uniform size. It is increased in the region supplying the wing musculature. A relatively greater increase would be expected in flying birds, the comparison of the ostrich with one of the large birds of prey would be interesting. When we go farther caudalwards and come to the increase of the primary apparatus corresponding to the massive leg musculature we find a great tumor-like enlargement, or Locomotor Brain, which demonstrates, as perhaps nowhere else in the animal kingdom, the close interdependence between a section of the central nervous system and the area innervated.

An interesting feature of the lumbo-sacral enlargement is the manner in which the neuromeres are marked off on the ventral surface of the cord by the hill-like prominences, calling to mind the segmental appearance presented by the well-known Trigla cord.

The marked development of the sinus rhomboideus offers favorable conditions for the study of this characteristic feature of the bird cord. We are enabled to contribute some facts as to the nature of the peculiar tissue with which this sinus is filled.

In studying the finer structure of the cord, the grouping of the cells into defined columns could be followed, some of which extend throughout the length of the cord. Two particularly interesting groups were found, one limited to the thoracic region in the posterior grey commissure, the other a group of "giant" cells occurring in the lumbo-sacral and cervical enlargements. The segmental groups of cells or nuclei occurring on the periphery of the cord, which have recently been the subject of much attention, are found in the characteristic way, and moreover are here present as macroscopic structures.

Our material was not such as to allow us to say anything of especial importance concerning the fibre tracts that would be new for the bird spinal cord. In this direction we can only look for advancement from experimental work such as was begun by Friedländer in this laboratory. Attention, however, is to be called to the short course taken by the fibres in the dorsal funiculi, and to the small proportion of these fibres that eventually reach the higher centers through this path directly.

THE DEVELOPMENT OF THE HYBRIDS BETWEEN FUNDULUS HETEROCLITUS AND MENIDIA NOTATA WITH ESPECIAL REFERENCE TO THE BEHAVIOR OF THE MATERNAL AND PATERNAL CHROMATIN.

BY

WILLIAM J. MOENKHAUS, PH. D.

WITH 4 PLATES.

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I. INTRODUCTION.

During the summer of 1899, while endeavoring to find two species of fishes that I could readily hybridize with the view of making certain variation studies on hybrids, I began what has since grown into a rather extensive series of experiments on the limits of crossing in fishes. Among many crosses effected, the one which proved of special interest both then and since, is that between *Fundulus heteroclitus* and *Menidia notata.* The results of the other crosses I have reserved for another paper. In the following pages only the results obtained on the above-named hybrid are considered.

In addition to their availability, the long period over which they spawn and the ease with which they can be hybridized, the reason for making a special study of the hybrids between Fundulus heteroclitus and Menidia notata, is the fact that the chromosomes of the two species can be readily distinguished morphologically. This fact is a distinct advantage in following out the nuclear history with reference to the im-

portant question of the individuality of the maternal and paternal chromosomes during the development of the hybrids. Before taking up this question, a brief description of the impregnation, cleavage and later development of these crosses will be given.

Among the many to whom I am under obligations for favors, I wish especially to mention Professor Charles B. Davenport, not only for much help during the progress of the work, but also for first directing my attentions to the possibilities in this line of experimentation. I wish also to especially thank the United States Commissioner of Fish and Fisheries, Geo. M. Bowers, for continual privileges at their Woods Hole Marine Station.

II. Material and Methods.

Fundulus heteroclitus and Menidia notata are among our most common coast fishes. Both species can be obtained in any desired number in the bays along our eastern coast. They spawn over a period of about six weeks, beginning the latter part of May. The spawning period must be about the same, since I have always been able to obtain ripe individuals of both species at the same time during the period above mentioned. The eggs of Fundulus heteroclitus are the larger, measuring 13-14 to the inch. I have taken as many as 599 eggs from a single large female, but the number obtained is usually considerably smaller than this. The eggs of Menidia notata measure on the average 26 to the inch. It is easy to get several hundred eggs from a single female. A rather large, well-filled female yielded, by actual count, 1413 eggs. The eggs of both species flow readily if properly handled. Those of Menidia often flow so easily as to make it difficult to handle a ripe individual without losing a portion of the spawn. Menidia notata has a much greater abundance of milt, so that it can easily be expressed as a thickish, perfectly white fluid. It is less easy to express the milt from Fundulus heteroclitus, so that I have usually found it preferable in my experiments to cut out the testes and tease them apart over the eggs. The two species belong to two distinct orders, Fundulus to the Haplomi and Menidia to the Acanthopteri.

The eggs were, in all cases, fertilized in small watch-glasses. All the eggs desired for any given experiment were first expressed into this watch-glass. Sometimes the eggs of a number of females were placed together when a large lot was desired. The milt was then added and after ten or fifteen minutes the contents were emptied into a finger-bowl of fresh sea-water. By a series of washings the excess of milt and the defective eggs were removed. The water was renewed two or three

times a day and the eggs were allowed to develop as far as they would. During the two-, four- and eight-cell stages the per cent of eggs impregnated and the character of the impregnation was determined. The normally impregnated eggs were isolated and their further development watched from time to time, and the desired stages preserved.

The necessity of proper precautions is, of course, evident, to prevent contamination by the introduction of other sperm than that desired. These, in all the experiments, consisted in (1) carefully sterilizing all the vessels and instruments that were used in the experiments; (2) keeping the two sexes of the same species in separate aquaria; (3) carefully washing the hands and the fish at the time of the experiment; and (4) carrying a control lot of eggs, taken from the same lot used for the experiment, in a separate fingerbowl containing water from the same source as that used on the eggs that were hybridized. I may say here, that in none of the experiments did I find a single egg of the control lots that showed any signs of development; so that there is no doubt that in all of the experiments the possibility of an error from contamination has been eliminated.

Not all the lots of eggs that may be obtained during the spawning season of a species will show the same per cent of impregnation, even with sperm taken from the same species. In order, therefore, to obtain a more reliable estimate of the percentage of hybrid eggs impregnated, it was essential in each experiment to fertilize a sufficient number of eggs from the same lot to serve as an index of the normal condition. Furthermore, it was most essential in determining whether the development of the hybrids was going on under favorable conditions, to carry along with them some normal eggs under the same conditions.

The developing eggs were kept under close observation from the time of impregnation to their death. Extensive notes were taken on the living eggs and desired stages were preserved in a variety of killing fluids—Perenyi's, Picro-acetic, Zenker's; and for surface study Child's method, of first placing the eggs for about a minute in a corrosive-acetic solution and then, after rinsing in water, in 10 per cent formalin, was used. This latter method in both species of eggs leaves the egg membrane and yolk beautifully clear while it turns the protoplasmic portions white, thus making an ideal preparation for surface study.

III. Nomenclature.

For clearness and brevity's sake in the following discussion I have found it desirable to adopt certain expressions which should here be

defined. An egg or embryo obtained by using Fundulus heteroclitus as the female, is designated as the Fundulus egg, embryo or hybrid, as the case may be. The reciprocal, with Menidia notata as the female, will then be a Menidia egg, embryo or hybrid. A normal egg, embryo or cross is one in which both parents belong to the same species, in distinction from a hybrid egg or embryo in which the two parents belong to different species.

IV. Fertilization.

1. *Fundulus heteroclitus, female, and Menidia notata, male.*

The cross in which Fundulus heteroclitus was used as the female was made ten times. In three of the experiments the males had died before the milt was taken. In one of these the male had been dead for an hour but the milt was normally white and the results of the experiments could not be told from those in which the males were alive and vigorous.

The per cent of eggs impregnated was not determined for all the ten experiments. In all the experiments, however, it was considerably above 50 per cent. Below are given the per cents based on actual count of four experiments. The per cents in these range from 70 to 93.

Experiment No. 24b	.....	87 per cent.
" " 25b	.....	80 "
" " 29b	.....	93 "
" " 126	.....	70 "

Of the eggs impregnated, approximately 50 per cent in each experiment were normally impregnated, the remainder were, with a very few exceptions, disperic.

2. *Menidia notata, female, Fundulus heteroclitus, male.*

The cross in which Menidia notata was used as the female was made 8 times. Two of these experiments were made at Cold Spring Harbor during the summer of 1898, and the remaining six at Woods Holl two years later. The results obtained at the two places were not the same. At the former place the per cent of eggs impregnated was very small, namely 14, while at the latter place the impregnation was nearly perfect, 96 per cent. The difference may be due to the fact that at Cold Spring the mother fish had to be transported for half a mile in a bucket, so that they were dead or almost so by the time that the eggs were procured. The experiments at Woods Hole were, on the other hand, carried on under the most favorable circumstances; the mother fish being alive

and vigorous at the time the eggs were taken. I think, therefore, that the eggs of Menidia notata can be almost perfectly impregnated by Fundulus heteroclitus.

The character of impregnation is in striking contrast to that of the reciprocal cross, in that practically all the eggs fertilized are normally impregnated. There was an occasional dispermic and some polyspermic eggs. This is true whether the per cent of eggs impregnated is small or large. None of these dispermic or polyspermic eggs were isolated to see how far they would develop, nor preserved for the study of their internal character. This difference in the character of impregnation in reciprocal crosses I have found nowhere so strongly marked in any of the many other crosses I made among fishes. The dispermic condition of 50 per cent of the eggs when Fundulus is used as the female, is regular and occurs in every experiment, so that this diffrence is a constant one.

At the time these experiments were made for the first time, I was not aware that any crosses between so distantly related species of fishes had been recorded. Subsequently I found that Appellöf, **94**, had made an equally remarkable cross between two European species: Labrus rupestris, female, and Gadus morhua, male. He says nothing about the percentage of eggs impregnated except that "ein Anzahl" were found in regular cleavage the following day, nor about the character of impregnation—whether any of the eggs were dispermic or polyspermic in addition to the normally impregnated ones. Pflüger's experiment, **82**, in which he succeeded in impregnating the eggs of an anuran, Rana fusca, with the sperm of a urodele, Triton alpestris and Triton tæniatus is in some respects even more remarkable. However, he succeeded in obtaining only polyspermic impregnation. Morgan, **94**, succeeded in impregnating the eggs of Asterias with Arbacia. Mathews, **02**, repeated the experiments and concludes that Morgan's impregnations were probably a species of parthenogenesis consequent upon shaking the eggs and not a true impregnation by the sperm of a sea urchin. My experience with many other crosses between fishes as distantly related as Fundulus and Menidia incline me to the belief that the normal impregnation of these two classes of Echinoderms is perfectly possible. This remarkable experiment deserves to be repeated with all possible precautions.

V. Development.

1. *Cleavage.*

a. Form of Cleavage.—The cleavage of the eggs normally impregnated goes on in a perfectly normal manner. The eggs all pass regularly

through the two, four, eight, sixteen, etc., cells which in no way differ from the corresponding stages of the normal eggs. The cleavage in the hybrid eggs might show differences (1) in the irregularities in the size of the cleavage cells or in the stages of different eggs in any given lot, and (2) in the rate of cleavage.

In a lot of Fundulus eggs, which have been taken from a single mother fish and normally impregnated, all the eggs, except in rare instances, will remain nearly perfectly abreast in their time of cleavage. I have observed, however, that in a composite lot taken from a number of females such perfect concert in the rate of cleavage may not obtain. If the eggs are impregnated by sperm from a strange species even so distantly related as Menidia this concert of cleavage is not affected so far as I am able to detect. The same can be said for the reciprocal cross. Three hybrid eggs came under my notice which should be mentioned in this connection. These had stopped in their development, the one at the two-cell stage and the other two in the four-cell stage. The blastomeres were in each case perfectly formed. The eggs were all found in the same lot. The three abnormal ones were isolated to watch their further fate. The eggs all died without dividing further. I have endeavored to determine whether there was a greater irregularity in the size of the blastomeres of the different cleavage stages, in the hybrids than in the normals. My observations go to show that this is not the case in the stages below the 32-cell stage. Beyond this I was unable to make any comparison on account of the complexity of the cell mass. In a lot of normal eggs of Fundulus there are always to be found a number of eggs in which there is more or less variation in the size of sister blastomeres. One cell in the 2-cell stage, in an extreme case, may be several times smaller than its mate. From this condition to that of perfect equality in the size of the blastomeres there are all intergradations. This inequality may be begun in the second or third cleavage where, in addition, the cleavage planes may vary considerably in their direction, giving rise to irregularities in the arrangement of the blastomeres. Such irregularities everyone has noticed who has watched any considerable number of cleaving fish eggs. In the hybrid eggs I was unable to make out any difference in the extent to which such irregularities occurred. This was certainly contrary to my expectations, since I had considered it unlikely that two different chromatins of such diverse origin would work so perfectly together through all the complicated activities incident to cell division. That this may be, is evident not only from the above consideration, but also from a closer study of the internal phenomena described further on and from similar observa-

tions on a great many other equally distant crosses which were made among fishes.

b. Rhythm of Cleavage.—In general, as already pointed out by Born, **83,** the hybrid egg develops slower than the normal. I have observed this repeatedly in fishes. In most of the crosses that have come under my observation, however, the difference in the rate is very slight and cannot in most cases be detected in the early cleavage stages. In the following table is given a comparison of a lot of hybrid eggs with a lot of normals. The eggs were taken from the same mother at the same time, fertilized at the same moment and kept under exactly similar conditions. The observations were made at the same time on both batches of eggs and the stage at which each was found was indicated as accurately as possible.

Time of Observation.	Fund. × Fund.	Fund. × Men.
9.10 P. M., June 26.[1]	In 2 cells.	In 2 cells.
9.40 " "	Beginning 4 cells.	Beginning 4 cells.
10.00 " "	Completion 4 cells.	Completion 4 cells.
10.15 " "	Beginning 8 cells.	Beginning 8 cells.
10.20 " "	Well begun on 8 cells.	Well begun on 8 cells.
10.30 " "	In 8 cells.	In 8 cells.
11.00 " "	Beginning 16 cells.	Beginning 16 cells.
9.00 A. M., " 27.	Well along in segmentation.	Well along in segmentation.
9.00 P. M., " "	Well begun on gastrulation.	First trace of gastrulation.
9.00 A. M., " 28.	2/3 + over the yolk.	½ or less over the yolk.
3.00 P. M., " "	Blastopore closed.	2/3 over the yolk.
5.30 " " "	Blastopore closed, the embryo long and narrow.	Blastoderm closing or nearly closed; embryo much shorter than normal.
9.00 A. M., " 29.	Embryo with optic vesicle.	Blastopore closed, embryos short, no optic vesicle; apparently dead.

[1] Eggs fertilized at 7 P. M., June 26.

From the table it will appear that the retardation in the development does not appear until the close of cleavage. If the development of the hybrid is slower than the normal during the first four or five cleavages it is so slight that it cannot be detected. From this time until the time of gastrulation the hybrids fall considerably behind. From the time of gastrulation on they fall increasingly more behind the normals. It is probable that the slowing-up process does not take place at the same rate but that it becomes increasingly rapid as development proceeds. In the reciprocal cross, if compared with the normal eggs of Menidia, the same conditions obtain. The normal Menidia eggs cleave a little

more rapidly than those of Fundulus. This increased rate is also maintained for the Menidia hybrid eggs.

This law of the rate of cleavage in hybrids I have considered elsewhere but the following facts are of interest here. When the two species crossed have eggs that cleave at a different rate the cleavage is still that of the egg species. The eggs of Fundulus heteroclitus can very easily be impregnated by *Tautoglabrus adsperus*. The eggs of the former cleave ordinarily in about two hours after the addition of the sperm. Those of the latter, under similar conditions, cleave in about fifty minutes. In the hybrid, however, the rapid sperm is unable to alter the rate of the cleavage and vice versa. This law is further strikingly illustrated in the cross between *Batrachus tau* and Tautoglabrus. The eggs of the former species can be impregnated by the sperm of the latter. The cleavage furrows, however, do not appear until 8 hours after impregnation, approximately that of the egg species.

Stassano, **83**, maintained that he was able to hasten or retard the cleavage of Echinoderm eggs by sperm of another species. Driesch, **98**, however, by extended experiments in the same group of animals, has shown just the reverse and it is probable that Stassano erred in his experiments.

2. *Development of Dispermic Eggs.*—The dispermic eggs fall at once into four cells. The cleavage takes place synchronously with the cleavage of the normal eggs, so that when the normal eggs are in the two-cell stage, about an equal number of eggs will be found in the four-cell stage. This correspondence in the rhythm of cleavage is not strictly maintained after the first cleavage, in that the rate is slightly slower in the normals. The form of the cell cannot be distinguished from those in the four-cell stage of the normals. The four-cell stage, or the first cleavage is followed by the eight-cell stage, this by the sixteen-cell stage, etc., in a normal manner.

Such dispermic eggs continue their development to a late stage of cleavage, when they invariably die. I have isolated, in the aggregate, many hundred dispermic eggs and followed their development but have never seen an egg that showed any signs of forming the germ ring or the embryonic shield. They form a normal heap of cells and the blastoderm may even spread to a slight extent, but beyond this they do not go.

That such eggs which fall at once into four cells are dispermic, i. e., eggs whose nucleus conjugates with two male pronuclei, is clearly shown

in sections of such eggs. Figure 1 (Plate I) shows the three pronuclei before their fusion. This egg would in all probability have fallen into four cells at once. In the metaphase there are two spindles placed at right angles to each other, an aster at each of their poles and at the point of intersection the chromosomes are being distributed. I am unable to say whether in such dispermic eggs more than two spermatozoa enter of which only two would then succeed in conjugating with the egg pronucleus.

Having such an easy way of producing dispermy I isolated large numbers of such eggs for further development to see whether I might be able to obtain any evidence on the question of the relation of double impregnation and double monsters or double embryos. Fol., **83,** was the first to raise this question in connection with his studies on Echinoderm eggs. He obtained from a lot of polyspermic eggs a considerable number of double and multiple gastrulæ. He maintained that the polyspermic condition was responsible for this result. In 1887 Oscar and Richard Hertwig reared many thousand polyspermic Echinoderm eggs and obtained only about ten double gastrulæ, a proportion entirely too small to lend any support to the hypothesis of Fol. Further observations of Oscar Hertwig, **92,** on isolated polyspermic frog eggs and of Driesch, **93,** on isolated dispermic Echinoderm eggs speak against this hypothesis. In the dispermic fish eggs I hoped that the double character might show itself, in the first place, in the double grouping of the cells in early cleavage and, in the second place, in the appearance of a double embryonic shield which could be taken as an indication of an attempt to produce two embryos. In regard to the first, it was found, as already intimated, that the early cleavage stages, so far as the form and grouping of the blastomeres are concerned, do not differ from the normal eggs. In regard to the second, none of the eggs went beyond the late cleavage stage. A careful search failed to reveal any sign of even a beginning of an embryonic shield. Inasmuch as the majority of the normally impregnated hybrid eggs develop far enough to form an embryonic shield and many of them considerably beyond, the fact that none of the dispermic eggs formed such shields must be taken as evidence against the theory of any relation between dispermy and double embryos.

3. *Later Development.*—When cleavage has well progressed in the Fundulus hybrid the blastoderm spreads and the germ ring with a faint indication of an embryonic shield, forms. From this stage on a variety

of conditions become apparent. The blastoderm may continue to spread in an apparently normal fashion, encompassing the yolk, and the embryonic shield enlarges correspondingly. The "blastopore" closes and the rudiments of the embryo are laid down. The three germ layers, the chorda and neural cord are differentiated (Fig. 2, Plate I). The eyes were seen in only two out of all the specimens that were obtained. Sections of one such embryo showed that the optic cup is forming and the lens, composed of a mass of cells, is constricted off from the ectoderm (Fig. 3). No definite arrangement of the cells in the lens can be made out. In the retina the cells were arranged into more or less distinct transverse rows. In both structures the cell boundaries are seldom to be made out; the nuclei, on the contrary, are large, distinct and provided with one or more very large nucleoli.

The proportion of embryos that thus normally close the "blastopore" is small. During the growth from the germ-ring stage to the closure of the "blastopore" the failures in the developmental processes especially show themselves in the variety of abnormalities which occur. The embryo may stop at the early embryonic-shield stage and, after two or three days of apparent life, die. Others endeavor to lay down the embryo so that the normal processes may go on for a time. The blastoderm may more or less completely enclose the yolk with the result that the embryos are too short in varying degrees and the "blastopore" may remain as a long slit or an open cleft of varying form (Figs. 4, 5, 6, 7, Plate I). The number of embryos dying at these varying stages is not the same. Comparatively few die during the early embryonic-shield stage. The bulk of the embryos starting on gastrulation succeed in encompassing the yolk to two-thirds or more of the extent giving rise to the variety of "blastopore" formations above described.

In the reciprocal hybrid the development beyond the cleavage stage is markedly less successful. A large per cent of eggs will form the germ ring and the early stages of the embryonic shield, but of these only an occasional one closes the "blastopore" in an approximately normal manner. The earlier stages of gastrulation not uncommonly form normally. Beyond this the abnormalities occur. These are of the same general character as those described in the Fundulus hybrid. Figures 8 to 10 (Plate II) show some typical cases.

In both hybrids the developmental processes come to a standstill at various stages during gastrulation and doubtless also during cleavage stages. That the latter is true is evident from the fact that there are always a number of eggs in cleavage that never form any germ ring. From the table given on page 35, it appeared that the rate of development of the hybrid eggs became increasingly slower than the normals as de-

velopment proceeded. This slowing-up process is to be interpreted as an increased weakening in the developmental energy. Either the unequal giving out of, or the unequal draft upon, this energy in different portions of the developing embryo, may result in the various abnormalities above described.

The bearing of these abnormalities upon the question of embryo formation is not to be discussed here. Other crosses have yielded material much more instructive and the subject will be taken up in connection with a description of those crosses.

VI. The Individuality of the Maternal and Paternal Chromosomes.

1. *Introduction.*—As stated in the introduction of this work, one of the points of especial interest in the hybrids between Fundulus heteroclitus and Menidia notata is the fact that the chromosomes of the one may be distinguished, morphologically, from those of the other. I was introduced into the importance of this through the study of a section of a hybrid egg which was in the anaphase of the first cleavage. In this spindle two kinds of chromosomes appeared, easily distinguishable. Subsequent comparison with the chromosomes of the parent species showed that one of the kinds of chromosomes belonged to one and the other to the other parent, and that the introduction into a strange egg did not modify their characteristic form. With these conditions obtaining it has been possible for me to follow the history of the maternal and paternal chromosomes in these hybrids to a late stage of cleavage. The phase of this subject which has engaged me especially is that of the individuality of the two parental chromosomes during development.

2. *Material and Methods.*—Appropriate stages were preserved from the moment of impregnation to a late cleavage stage. Corresponding stages of both hybrids and of normal eggs were taken. The killing fluids used were Flemming's, Zenker's, Perenyi's, and picro-acetic. The last two have been of most service to me. The eggs were directly placed into the fluids without first removing the membranes. The most convenient method for manipulating the eggs in the paraffin and one which I adopted altogether is as follows: the membrane was removed and the yolk with the protoplasmic cap or embryo surmounting it was imbedded with the cap directly upward. By properly mounting the paraffin block I could lay the protoplasmic cap into horizontal sections until the yolk was reached. This very much simplified an, at best, very laborious task. The sections were in practically all cases made 7½ or 10 micra thick. It

was found that such sections might include the entire thickness of the spindles in the earlier stages of cleavage. This was a matter of considerable importance since it was desirable to get all of the chromosomes of a given spindle in the same section. All the staining was done with Haidenhain's hæmatoxylin. This stain was found perfectly satisfactory, and because of its simplicity and the ease with which it can be controlled was used exclusively.

3. *Description of the Chromosomes.*—The chromosomes of Fundulus heteroclitus are long, slender and usually straight. They measure 2.18 micra in length. In a given anaphase all the chromosomes are of practically the same length. Just before breaking up into the chromosomal vesicles the constituent chromomeres can commonly be made out. These number four in nearly all cases that I have counted. In one instance one of the chromosomes had five. The number of chromosomes is 36.[2] In their migrations to the poles they lie alongside of each other parallel, for the most part, with the spindle fibres so that at the anaphase their form can be easily made out (Fig. 11, Plate II).

The chromosomes of Menidia notata are short and usually more or less curved. They are sometimes straight and in some cases slightly sigmoid. They measure 1.00 micron in length. As in Fundulus, they have a uniform size in any given anaphase (Fig. 12, Plate II). I have tried to make out the component chromomeres, but without success. The number of chromosomes is about 36. I have not been able to count them definitely.

In Figure 13 (Plate II), are given the two kinds of chromosomes drawn to the same scale. Both the relative size and the difference in form are shown. The difference in the two chromosomes comes out very strikingly also when seen side by side in the same cell [Figs. 14, 15 (Plate II) and 29, 30 (Plate IV)]. The small chromosomes are here grouped together on one side of each half of the spindle and the long ones on the other side of the spindle.

There can be no doubt that these two diverse forms of chromosomes occurring in the hybrid eggs are the chromosomes of the two diverse parents which, notwithstanding their association with strange chromosomes in a strange cytoplasm, are evidently functioning in a perfectly normal manner. A description of their behavior from the time of conjugation to a late cleavage stage is the purpose of the present section. Before en-

[2] In only one instance was I able to count the number satisfactorily. Every chromosome was definitely distinguished and counted in this case. I had, however, concluded that 36 was the approximate number from numerous counts made on cross-sections of anaphase spindles.

tering upon this, however, for reasons to be stated afterwards, it will be important to give a brief general account of the character of the work thus far done on this subject.

4. *General Review of Literature.*—Since the first discoveries by van Beneden, **83**, Boveri, **90**, Guignard, **91**, and others, of the numerical equality of the maternal and paternal chromosomes in fertilization, much interest has developed in the question whether these might not retain their individuality throughout all the cells of the developing embryo. Van Beneden, who worked with Ascaris, in which the pronuclei may not fuse before the formation of the first cleavage spindle, was able to follow the maternal and paternal chromosomes into the resting nucleus of the first two daughter cells. Here they were lost. Although unable to follow them beyond the first cleavage, he expresses his conviction that the two chromatin masses probably remain distinct throughout subsequent divisions. Boveri, **91**, working with the same animal, made similar observations and was led to formulate his well-known hypothesis "that in all cells derived in the regular course of division from the fertilized egg, one-half of the chromosomes are of strictly paternal and the other half of maternal origin." He further endeavored to follow out the fate of the individual chromosome during the resting period of the nucleus. Boveri differed from van Beneden in this important respect, that he found that not only the maternal and paternal chromatin remained distinct but also that the individual chromosomes retained their individuality. With this interesting and important question thus clearly pointed out so long ago, one should consider it remarkable that so few researches have since been directed toward its solution, were it not for the evident difficulties attending any effort to distinguish the exactly similar parental chromosomes beyond the first cleavage. Extension of our knowledge to a large number of forms showed that three conditions obtained in regard to the fusion of the pronuclei during fertilization: (1) Animals in which the two pronuclei are so completely fused as no longer to be distinguishable. (2) Animals in which the pronuclei do not fuse but remain more or less separated by a membrane. (3) Animals in which both conditions may occur.

In 1892 Häcker pointed out that in Cyclops tenuicornis also, the two pronuclei do not fuse in fertilization and, furthermore, that in the two-cell stage the nuclei are composed of two closely united but distinct halves, one of which he identifies with the male, the other with the female pronucleus.

Rückert, **95**, extended these observations to Cyclops strenuous and pub-

lished the first research directed specifically toward the solution of this question. Rückert found, in the first place, that the condition of double-nuclei could be followed considerably beyond the late cleavage stages and, in the second place, that the chromosomes might be arranged in two groups upon the spindle, more or less distinctly separated. From the bilobed nuclei of the one, two, four, etc. cells a double group of chromosomes might arise and these two groups could be followed each into one of the two halves of the subsequent resting nucleus. Such bilateral grouping of the chromatin in the spindle occurred only in the earlier cleavage but the double nuclei could be found, although in constantly decreasing number, in later stages. The strong probability that in the early stages the two halves of the double nuclei represent the double source of the chromatin, makes the assumption that in the later stages such double nuclei have a similar significance, justifiable. It is worth while to state in this connection Rückert's cautious conclusions in his own words, "Jedenfalls geht aus der vorstehenden Untersuchung hervor, dass in der ersten Entwickelungszeit mindestens bei einem Theile der Kerne eine Vermengung der väterlichen und mütterlichen Hälfte nicht statt hat, das ein solcher Vorgang für den normalen Verlauf der Entwickelung somit nicht erforderlich ist. Das Chromatin kann seine ursprüngliche Vertheilung beibehalten trotz wiederholter mitotischer Theilungen und Auflösungen in ein feinfädiges Gerüst, und obwohl die übrigen Lebensvorgänge innerhalb seiner Substanz, die Assimilation und das Wachsthum, gerade zu dieser Zeit der rasch aufeinanderfolgenden Theilungen lebhaftere sind als sonst."

V. Häcker, **95, 02,** working also on Cyclops, carries the observations of Rückert considerably further. In addition to the double nuclei and the bilateral distribution of the chromatin on the spindle, he observed a physiological difference in the maternal and paternal chromatin masses. This physiological difference showed itself in the different stages in which the two masses of chromatin may be within the same cell. It enabled him often to distinguish two groups when otherwise there was no spatial separation or no nuclear membrane to separate them. In Cyclops brevicornis, however, he could not recognize the double distribution of the chromatin beyond the eight-cell stage except in the primordial germ cells from the beginning of gastrulation.

Recently, Conklin, **01,** has shown that in Crepidula, even more clearly than in Cyclops, the double character of the nuclei during certain phases quite commonly obtains during earlier cleavage (first 5 or 6 generations), and gives this the same interpretation that others had given it.

Conklin called attention to the fact that in these double nuclei each

half usually contained one nucleolus, so that these might also be regarded as maternal and paternal. Häcker, **02**, in a recent preliminary, summarizes the results of his comparative study of species of Cyclops. He endeavors to show that the maternal and paternal chromatin masses are each represented by a nucleolus in certain phases of the cell cycle. Taking these as an index he is able to establish the individuality of the parental chromatin masses throughout the cycle of an individual.

Among plants Miss Ferguson, **01**, has shown that in Pinus strobus the male and female chromatin remains distinct during the first two cleavages, as far as she has followed them, and suspects from sections of later stages that this individuality may persist.

The two important papers by Herla, **93**, and Zoja, **95**, on Ascaris hybrids treat the subject from a standpoint so similar to that of my own work that it will be advantageous to consider them later.

The above brief survey of the work thus far done on this subject enables me to point out the following facts: (1) The evidence upon which the authors base their conclusions rests on the assumption that the two halves of the double nuclei occurring beyond the two-cell stage represent, the one, the maternal, and the other, the paternal chromatin. (2) The chromatin of the two parents not only retains its individuality but also remains spatially separated or bilaterally distributed in the nucleus during the various phases of division. In the following detailed consideration of my own results I shall have occasion repeatedly to refer to these facts.

5. *Conjugation of Pronuclei and the First Cleavage.*—The time elapsing between the moment of entrance and the time of conjugation of the pronuclei is the same as that for the normal eggs. Thus, in the Fundulus cross, at a temperature at which the first cleavage furrow forms just two hours after impregnation, the male pronucleus has become apposed to the female pronucleus at 55 minutes after fertilization. At 65 minutes, they have usually become well fused. During its migration the sperm has become metamorphosed into a vesicle which cannot be told from the female pronucleus. I have taken much pains to find some distinguishing mark between the maternal and paternal chromatin at this stage, hoping such might serve in distinguishing them in subsequent resting stages. The size, form and arrangement of the chromatin granules in the two pronuclei, however, are, so far as I have been able to make out, altogether similar.

In a late metaphase of a Fundulus hybrid egg, 73 minutes after fertilization, two chromatin masses can readily be made out (Fig. 16, Plate II). The one is evidently made up of the long chromosomes which, in

the process of splitting, already extend their free ends for some distance on each side of the equatorial plane. The other group is made up of the short chromosomes which, also in division, appear as large granules rather than elongated structures.

In the anaphase [Figs. 14, 15 (Plate II), 29, 30 (Plate IV)], two groups of chromosomes occupy each half of the spindle. Their form here comes most distinctly to view. Careful examination of each group shows that it comprises chromosomes of only one type, so that the chromatin material has not become mingled during the fusion of the pronuclei. The two groups do not occupy the same position with reference to the pole or the equatorial plane, i. e., they are not equidistant. In other words, the two kinds of chromosomes are not in the same stage of migration. This physiological difference is not so well marked in the Fundulus hybrid where the small ones become the stragglers [Figs. 14 (Plate II) and 29 (Plate IV)], but is very distinct in the Menidia hybrid [Figs. 15 (Plate II) and 30 (Plate IV)].

As the chromosomes become transformed into the resting nucleus each is converted into a vesicle in a manner essentially similar to that described for Crepidula by Conklin. In an early stage the two groups of vesicles can be distinguished by the difference in the size of the vesicles. These fuse at first into larger ones, giving rise to a lobed nucleus. At this stage it is no longer possible to tell the two kinds of vesicles apart. The fusion continues until a single well-rounded resting nucleus results, with all traces of its double character lost.

6. *Second Cleavage.*—Although all traces of the maternal and paternal chromatins are lost in the resting nucleus of the two-cell stage there is very little doubt that they have really remained spatially distinct. That this is so, is shown by the fact that when the chromatin forms into the chromosomes of the next cleavage the two kinds again appear, and in all the spindles examined they were again bilaterally distributed on the spindle.

The kinds of chromosomes can, naturally, be best distinguished during the anaphase, but even in the metaphase this may be done. In such a metaphase of a Menidia hybrid, for instance, Fig. 17, where the long chromosomes are the introduced ones, there may be seen on the one side of the spindle the long ones with the ends of a part of the chromosomes already extending toward the poles, while on the other side the short ones may be seen confined with characteristic strictness to the equatorial plane. Figures 18 (Plate II) and 31 (Plate IV) represent one of the groups of the migrating chromosomes of an anaphase of a second cleavage. Here the chromosomes come most distinctly to view. The

long ones all grouped together on one side of the spindle (left in figure) and the short ones on the other side. Whether all of each kind that entered the resting nucleus have again appeared I cannot say, inasmuch as it has been impossible thus far to recover all the chromosomes of each parent. This is due to the complexity of the chromosome mass. The chromosomes are so small and numerous that their number cannot be determined even in the clearest preparation. In any given section some of the long chromosomes are usually cut, making the pieces indistinguishable from the short ones, so that it is practically impossible to follow out all of the chromosomes of each kind. That we have here to do again, however, with the maternal and paternal chromosomes, there cannot be the shadow of a doubt.

Rückert, 95, in his Fig. 6, gives the lateral view of the anaphase of the second cleavage spindles of Cyclops strenuous, both of which, but especially the spindle to the right in the figure, show two groups of chromosomes spatially separated in each half of the spindles. Rückert takes these to be the maternal and paternal groups of chromosomes, and he gives, it would seem, very good reasons for thinking so. That the two chromatin masses of the first cleavage represent the two parental chromatins is beyond question. He is able to follow them from the time of the conjugation of the two pronuclei through the various phases of the division to the reconstruction of the resting nucleus. In this reconstruction the first appearance of the nucleus is that of a double group of small vesicles. The vesicles of each group, it appears, fuse with each other, the halves remaining distinct at first by the presence of a more or less distinct wall and a corresponding constriction in the outer membrane but later only by the latter. The two halves of the resting nucleus, therefore, are to be identified as the maternal and paternal portions. The emergence of two chromatin masses from this double nucleus in the following division distributed on the spindle in the manner above described, would strongly favor the view that the two substances had not mingled during the resting stage. Conversely, the strong probability that the two chromatin masses remain distinct in the previous resting nucleus argues strongly in favor of Rückert's supposition that the two groups of chromosomes appearing in the subsequent division represent the maternal and paternal chromosomes. Conklin has given very strong evidence for the same thing in Crepidula. It is apparent to every one who has closely followed through the researches described above that the one thing to be desired is better evidence that the two groups of chromosomes emerging from the two bilobed resting nuclei of the first two blastomeres are derived from the two lobes of the nuclei

and really represent the maternal and paternal chromatin. Conklin expresses the situation in the following words: "It still remains to show that these double nuclei really represent the egg and sperm nuclei which have not yet lost their individuality. This cannot be demonstrated in Crepidula, for the reason that this double character is not apparent at every stage in the nuclear cycle, but it is extremely probable, as the following observations will show:" The detailed reasons given need not be repeated here.

There are but two ways to demonstrate this with certainty, namely, either to follow the process in the living egg, or to be able to distinguish the two kinds of chromosomes, as I have been able to do in the hybrid under consideration.

Herla, **93**, and Zoja, **95**, made some observations which bear directly upon this point. In the study of an Ascaris containing eggs in various stages of early cleavage they found that the number of chromosomes in the cells was only three, one of which was slightly smaller and like the chromosomes of the variety univalens. The eggs, they conclude, were hybridized by the sperm of univalens. They were able to trace the independent maternal and paternal chromosomes to the 12-cell stage. With only three chromosomes it is not possible to determine with certainty very much about their distribution in the spindle. Zoja, in fact, says that the small chromosome may vary its position with reference to the other two, sometimes being between the two latter. These observations, therefore, can throw little light on the particular question of the distribution of the two chromatins in the nucleus.

In my own hybrids, however, where the number of chromosomes is great, any disturbance of their grouping can be readily made out. The conditions described for these hybrids, taken in connection with the observations of Herla and Zoja, demonstrate in the clearest manner that the two chromatins may remain distinct in these resting nuclei and that the chromosomes in the subsequent division may be and are grouped spatially. They, furthermore, lend the strongest support to the belief that in the other forms described (Cyclops, Crepidula, Pinus) the two groups of chromatin or chromosomes arising from the bilobed resting nuclei of the two first blastomeres may really represent the distinct parental chromosomes.

7. *The Rotation of the Cleavage Nuclei During the First two Cleavages.*—In the first cleavage spindle the chromosomes lie side by side in a horizontal plane. In this same plane they can be followed into the early resting stage of the two daughter nuclei. Evidently, inasmuch as the second cleavage plane forms at right angles to the first, the nucleus will

have to rotate through an arc of 90° in order that both kinds of chromosomes may again be halved. This rotation takes place between the vesicular stage of the nucleus and the metaphase of the following division. Just when during this period it takes place or mostly takes place I cannot say. At the metaphase the rotation is probably completed. When the rotation is completed both chromosome groups again occupy a horizontal plane.

In all but one of the cells of the Menidia hybrid examined the small chromosomes bore a definite relation to the cleavage plane, that of a position in the spindle away from the plane of division. In the single exception it was found that the small chromosomes were above the large ones and, hence, occupied a vertical plane.

The behavior of the chromosomes in the third and subsequent cleavages is different from that of the first two. It will, therefore, be advisable to describe these stages in detail before entering into a comparison with the conditions found in other forms.

8. *Third Cleavage.*—The two groups of chromosomes of the second cleavage spindles pass into a resting condition of the four-cell stage, in which it is again impossible to distinguish the two kinds of chromatin. There is no constriction or partition to divide the nucleus into two lobes or parts, as is common in Cyclops and Crepidula. Since, so far, the chromatin in these hybrids had behaved essentially like that in the other forms described by other authors, I expected that in the metaphase and anaphase of the third division the short and long chromosomes should again appear in two groups on the spindle. If the conditions here should run parallel with the conditions in Cyclops and Crepidula, this is what should be expected. But in this I was disappointed. Whereas in the second cleavage every cell which I examined shows the two kinds of chromosomes bilaterally distributed, the third cleavage spindles, for the most part, do not show such distribution. An occasional spindle occurs in which the grouping has not been completely destroyed. Figure 19 (Plate III), it will be seen, shows the short chromosomes to the right and the long ones largely to the left. It is to be noted, however, that each kind is not restricted to its group but a few of each kind have become mingled with those of the other. The mingled condition is the prevailing type where it is impossible to make out any grouping.

The position of the parental chromosomes with reference to the cleavage plane which could be so readily followed out during the second cleavage is during the third cleavage, naturally, largely destroyed since the bilateral distribution of the chromatin has been destroyed. In three spindles in which the position could be made out with reasonable cer-

tainty the small chromosomes were placed in a horizontal plane toward the side away from the last cleavage plane.

9. *Fourth Cleavage.*—When in the fourth cleavage the chromatin has resolved itself into chromosomes the two kinds are again mingled. The mingling has evidently gone farther, because in very few of the cells can even a partial grouping be discovered. I have found only one cell in which the two kinds of chromosomes were bilaterally distributed upon the spindle. In the sectioning of this cell the knife cut in such a way as to pass between the two groups so that in the one section nearly all short ones were found, and in the other section nearly all long ones. In my study of these sections I had well in mind the possibility that the short ones might be the ends cut from the long ones of the other section. That this is not the case, however, is evident from the fact that the long chromosomes of the one section have the characteristic length of the one species, and those of the other section that of the other species. There cannot be any doubt that we have to do here with two kinds of chromosomes, and that we can be perfectly certain these represent the distinct maternal and paternal groups. The usual condition is for the chromosomes to be well mingled on the spindle. In such an anaphase, Fig. 20 (Plate III), the two kinds of chromosomes can be clearly made out. In endeavoring to recover all of the chromosomes of each kind, I have found it convenient to draw each chromosome as I followed it, retaining its relation to some other one or more chromosomes but not its position in the spindle. Figure 21 (Plate III) represents such a drawing of an anaphase of the fourth cleavage. Although, as stated above, I have been unable to recover all the chromosomes, the drawing which I made as faithfully as I could with only the partial aid of a camera, serves well to show the presence of two kinds of chromosomes and their mingled condition.

10. *Later Cleavage.*—I have followed the behavior of the maternal and paternal chromosomes from the fourth cleavage through successive stages to late cleavage. Here, with often several hundred cells in any given section, in all stages of division and cut in many different planes, the conditions for such study are favorable. I have carefully examined many thousand cells in both hybrids with the view of finding one in which the two kinds of chromosomes had remained grouped but I have not been able to find a single undoubted instance. On the other hand, nuclei showing the two kinds of chromosomes mingled together upon the spindle are everywhere to be found. The two kinds of chromosomes, naturally, cannot be distinguished in the metaphase, not even when the chromosomes have begun to split. In the stage represented in Figure 32 (Plate IV) (lower cell to left), some of the long chromosomes may be seen

characteristically extending their ends toward the poles. The short chromosomes still confined to the equatorial plane cannot be identified as such. In nearly every anaphase, however, the short chromosomes can be clearly distinguished among the long ones [Figs. 22 (Plate III) and 32 (Plate IV)].

The two kinds of chromosomes can be distinguished not only by their size but also physiologically. In Figures 22 and 32, it can be seen that the short chromosomes, as a whole, are nearer to the pole than the long ones. This shows most clearly in the further half of the spindle where the short chromosomes remain more abreast and are, as a whole, nearer the pole, forming a band across the spindle. Here, as in the earlier stages of the Menidia hybrid, the long chromosomes are the stragglers, being more irregular than and, as a whole, behind the short ones in their migration to the poles.

While this difference in the rate of migration comes out most strikingly in the spindles where the chromosomes have not yet become mingled [Figs. 29, 30, 31 (Plate IV)], it is just as truly present in the later cleavage cells where this mingling has taken place. The small chromosomes, in the more extreme instances, may in the telophase become completely separated from the long ones, as shown in Fig. 23 (Plate III). This figure represents the early telophase of the third cleavage. The group of small vesicles nearer the pole are doubtless the small chromosomes already well along in their transformation while the larger group would then represent the larger chromosomes not yet so far along in their transformation but that some of the longer chromosomes can be identified. It will occur to every one that notwithstanding the fact that the chromosomes may be thoroughly mingled during the active phases of the cell cycle, the two kinds may in this way become separated in the resting nucleus. The reasons for believing that this does not usually occur will appear below in connection with another matter.

I have considered it important to carefully compare the conditions in the hybrids with corresponding stages in the normal eggs of the two parent species. Although practically all of the points above brought out would be sufficiently evident taken by themselves they become doubly so through such comparison. The question to arise is whether the differences in the size of the chromosomes might not also be found in the normals. This is clearly not the case. In any given cell during a phase when the chromosomes come distinctly to view all the chromosomes are of practically the same size. Any variations in their size cannot be confounded with the differences obtaining in the hybrid eggs. Furthermore, the chromosomes, apart from their size, show a certain individual-

ity in their behavior during various phases of division. In the equatorial-plate stage the chromosomes of Menidia notata arrange themselves in an even band across the spindle. In a corresponding stage in Fundulus, the plate presents a more ragged appearance, the ends of some of the chromosomes extending out towards the pole. This difference is especially well marked just at the time of splitting. In Figures 24 (Plate III) and 33 (Plate IV) is shown a cell in later anaphase of Menidia notata taken from about the middle cleavage stage of the embryo. The chromosomes are in a compact group without any stragglers along the spindle as is so common in the hybrid cells. Figure 25 (Plate III) is taken from an early cleavage stage of Fundulus heteroclitus. All the chromosomes are of the characteristic long form. In the later anaphase, [Figure 26 (Plate III)], corresponding approximately to the stages above given for Menidia notata (Figs. 24, 33), these chromosomes retain their characteristic length and extend along the spindle for some distance. If with these conditions in the normals corresponding stages in the hybrid are compared (Figs. 22 and 32), it will be seen that in the latter the conditions characteristic of both species are present. Here, as already stated, the short chromosomes appear as a band nearer the pole, extending across the spindle, and the longer ones, belonging to Fundulus, extend further along the spindle toward the equator. This somewhat tardy migration of the longer chromosomes may be caused by their not being in their native cytoplasm, for in the reciprocal cross where the conditions are reversed this difference in the rate of migration does not obtain.

11. *Comparison with Other Forms.*—When I first discovered that Menidia and Fundulus possessed two kinds of chromosomes and that these could be distinguished in the first cleavage spindle, I went at once to later cleavage stages for the further study of their behavior. In such stages I could easily get great numbers of cells in all stages of division in a single section. Those who had worked upon other forms had found that even in late cleavage stages the double nuclei, representing the maternal and paternal chromosomes, were more or less abundantly present. In sections of such stages I found no difficulty in finding evidences of the kind that had been employed by others in their studies upon other forms, namely, the grouping of chromosomes into two groups, during various stages of the division of the cell, and bilobed and double resting nuclei and the rather constant presence of double nucleoli in each nucleus (Fig. 27, Plate III). Knowing that the cells contained chromosomes of such different form as I had seen in the first cleavage, I was much disappointed at my inability to identify the two kinds here. In those cells where the chromosomes were grouped I had every reason to expect the

one group to show one kind, and the other group, the other, but just this I was unable to do satisfactorily. One of two things must be true, (1) either the distinction between the two kinds of chromosomes had disappeared or (2) they had become mingled in the course of development. The fact that I could make out two kinds of chromosomes which, however, were mingled upon the spindle, spoke directly for the latter view, but I still hoped that a study of successive stages from the first cleavage on might enable me to find conditions here similar to that found in other forms. As already indicated, they found in Cyclops and Crepidula that the maternal and paternal chromatin remained not only distinct but also spatially separated up to varying late stages of development. Except during the first and second and, in part, the third cleavage, this condition does not obtain in the hybrids under consideration. The chromosomes become mingled. This mingling probably has begun to a slight extent in the second cleavage and is clearly well along in the third. By the time cleavage is well along all the somatic cells have them mingled.

The evidence that has been given to show that the two kinds of chromatin remain spatially distinct in the forms referred to above is very strong. I have shown beyond any doubt that this may be the case for a very brief period in development. It is possible that the length of this period may differ in different forms even to the extent that they remain thus distinct throughout the entire embryonic period. It is interesting in relation to this subject to compare the results of Rückert on Cyclops strenuous and that of Häcker on Cyclops brevicornis. The former found double nuclei, although in constantly decreasing number, up to a late stage of development. The latter no longer found these double nuclei in the 16- and 32-cell stage nor in the stage just preceding the migration of the sex cells to the interior. He was, however, able to distinguish the two masses by physiological differences in the sex cells. This shows what a difference may obtain in two species of the same genus.

In the Menidia and Fundulus hybrids the bilateral arrangement of the chromosomes is destroyed at about the same stage as in Cyclops brevicornis, namely, at the third and fourth cleavage. According to Zoja 95, the two kinds of chromosomes in the Ascaris hybrid may be mingled before the 12-cell stage. These observations suggest that possibly the reason Häcker could not find the double nuclei beyond the 16-cell stage lay in the fact that in Cyclops tenuicornis the chromosomes also became mingled early in cleavage. Rückert did not find any distinct grouping of the chromosomes during the active phases of cell division beyond the four- and eight-cell stage. In the light of Häcker's observations on a

form so nearly related to Rückert's and that of Zoja's and mine, it may well be questioned whether the double and bilobed nuclei of Rückert really are any indication of the distinctness of the maternal and paternal chromatin. These conditions should at least make us cautious against accepting too readily the conclusions based on the mere presence of double and bilobed nuclei, double nucleoli and the like without any further means of identifying them with the maternal and paternal chromatin. Further work on a large number of forms is desirable to see whether it is the rule for the parental chromosomes to become mingled in these early cleavage stages.

12. *Maternal and Paternal Nucleoli.*—The recent studies of Häcker, 02, on parental nucleoli in different Copepod crustacean forms, has already been mentioned. The rather constant presence of two nucleoli in the nucleus he takes as an index of the separateness of the maternal and paternal chromatin. According to this idea, one of the nucleoli would represent the chromatin of the one parent and the other that of the other. At the time that his preliminary paper appeared I had been working on the nucleoli in fish hybrids. Most of the nuclei in the resting stage, when not too young, show two nucleoli. In the reconstruction of the nucleus the smaller chromosomal vesicles at first fuse into larger ones. In this stage one can often see a number of nucleoli in each nucleus. This multinucleolate condition is followed by a binucleolate condition as the fusion of these larger vesicles is finally completed. Each vesicle seems to form a nucleolus so that the number of nucleoli present in a nucleus is in a general way an index of the number of vesicles composing the nucleus. Observations of this kind, it seems to me, strengthen Häcker's position. Two nucleoli would indicate that the nucleus is essentially composed of two vesicles or units of some kind, although these could not be distinguished in any other way. I have endeavored to make out some constant difference in the size or structure of these nucleoli in the hybrids but without success. In cells of the same section all conditions obtain in the size, from a strongly unequal to perfectly equal nucleoli within the same nucleus. This interpretation of the nucleoli by Häcker has much in its favor. In such forms as he studied, in which he maintains that the two parental chromosomes remain bilaterally distributed, it is easier to conceive how the nucleoli might represent the two paternal chromatins. In the fish hybrids under consideration, however, where the binucleolate condition is probably just as constant for the cells as in Cyclops, but where I have shown that the two chromatins do not remain bilaterally distributed but both kinds are scattered through the nucleus, it is difficult to believe that the scattered chromosomes of a given parent are represented by a common nucleolus.

13. *The Persistence of the Individual Chromosome.*—The question whether the individual chromosome persists through the resting stage so that upon the resolution of the reticulum into the chromosome the same component chromatin granules again go together to make the same chromosome from which they were derived is a question first raised by Rabl, **85,** and later definitely stated by Boveri, **88.** Since that time so much evidence has accumulated going indirectly to support this conclusion that it has come to be rather generally accepted. Even a general review of this evidence is unnecessary here. Such a review would show that the fact has never been definitively demonstrated. Some of the most direct evidences yet given are the observations of Herla, **93,** and Zoja, **95,** on the Ascaris hybrids in which it was shown that the small chromosome of the variety univalens which entered the resting nucleus with the larger ones of the variety bivalens again emerged in its characteristic form. Equally strong evidence is now afforded by my own observations on hybrid fishes. Here, as in the Ascaris hybrids, two kinds of chromosomes enter the resting nucleus from which each kind again emerges. As long as the two kinds remain grouped, as during the first two divisions, this fact has little added significance, since within each group it would be perfectly possible for the component chromosomes to exchange chromatin granules during the resting period. If, however, as occurs in later cleavage, the two kinds of chromosomes become mingled the chromatin granules of both kinds must lie mingled together within the resting nucleus. If from such a nucleus the two kinds of chromosomes again emerge it amounts almost to a demonstration that the chromatin substance of a given chromosome forms a unit and that this unit persists.

It should be mentioned here that the hypothesis of Boveri of the independence of the parental chromosomes has not received universal support. Prominent among those who have held in varying form the opposite view, namely, that the two parental chromatins become fused and mixed either at the time of fertilization or during development, is Hertwig. Hertwig, **87,** maintained that fertilization demanded the thorough mixing of the sperm chromatin with the egg chromatin. Later, **90,** he revised his view in that he no longer considered it essential that this fusion takes place at the time of fertilization but that it nevertheless took place later, during the earlier stages of development. Wilson and Mathews, **95,** from their studies on the fertilization of Echinoderm species, concluded that because the fusion of the two pronuclei is here so thorough it would be impossible to maintain that the two chromatin masses remained distinct.

These objections have largely been disposed of by the researches of Rückert, Häcker, Herla, Conklin and others. These leave little doubt that the maternal and paternal chromosomes may remain distinct to a late stage in development, and I have shown that however thoroughly the chromosomes may lose their identity to our view during the resting period of the cell they nevertheless retain their individuality.

In view of its possible bearing on the theories of heredity just now becoming prominent through the recent rediscovery of the Mendelian laws of inheritance, it is highly desirable that this question of the individuality of the parental chromosomes be most thoroughly investigated. Further observations along this line on other hybrid fishes I have well under way, which I hope to be able to present in the near future.

SUMMARY.

The eggs of Fundulus heteroclitus can be readily impregnated with the sperm of Menidia notata. From 70 to 93 per cent of the eggs are fertilized. Of this number about 50 per cent are dispermic, the remainder, normal.

The eggs of Menidia notata can be even more completely impregnated by the sperm of Fundulus heteroclitus. Under favorable circumstances 96 per cent of the eggs are fertilized. Of these only a few are dispermic or polyspermic.

The normally impregnated eggs of both crosses develop normally to varying stages of embryo formation. They never go beyond the closure of the "blastopore."

The embryos differentiate the three germ layers, the chorda and neural cord. In rare instances the eyes may begin to develop—the optic cup and the lens being formed.

The per cent of eggs that develop to the closure of the "blastopore" is comparatively small. The per cent is much greater in the Fundulus hybrids than in the reciprocals.

The more usual thing is for the embryos to show abnormalities. These appear during the process of gastrulation and are probably all the expression of a weakening of the developmental energy.

The abnormalities take the form of variously shortened embryos with the "blastopore" completely closed or imperfectly so, in which case the latter may take the form of a long slit or of a cleft of varying irregularity in shape.

The early cleavage stages are passed through in a perfectly normal manner. The blastomeres show no greater variation in form from the typical than do normal eggs.

The rhythm of cleavage is that of the egg species. A spermatozoan from a species that normally has a different rate of cleavage cannot modify the rate of the hybrid egg.

Hybrid eggs may develop more slowly than normal eggs. This usually does not appear until later stages. As development proceeds the difference in rate grows increasingly great.

Dispermic eggs fall at once into four cells of the normal size and arrangement. This is followed by a normal 8, 16, 32, etc. cell stage.

The dispermic eggs of the Fundulus hybrid may develop to a late cleavage stage but never form a germ ring or embryonic shield.

The chromosomes of the two parent species, Fundulus heteroclitus and Menidia notata, are morphologically distinguishable, the rods of the former being long and straight in form, those of the latter, shorter, and commonly slightly curved. These retain their characteristic form when introduced into a strange egg through hybridization.

During the development of the hybrids they retain their individuality. During the first two cleavages each kind remains grouped and bilaterally distributed on the spindle. During the resting stage of the four-cell stage the chromatin becomes more or less mingled, so that when the third cleavage spindles are formed the grouping and the bilateral distribution of the chromatin has largely disappeared. During the following resting period the mingling has gone further, so that a complete grouping of the two parental chromosomes occurs very rarely in the following division. During the subsequent cleavages to a late cleavage only the mingled condition was observed.

This mingling of the chromosomes does not destroy their individuality for in stages of division favorable to bringing out the form of the chromosomes both kinds can be readily seen.

In these hybrids any nuclear conditions which would indicate that the chromatin is bilaterally arranged does not indicate any bilateral distribution of the two paternal chromatins in those nuclei.

The mingled condition of the maternal and paternal chromosomes in all but the very early stages of cleavages in these hybrids makes the bilateral distribution in the other forms described—Ascaris, Cyclops, Crepidula and Pinus—an open question.

The conditions obtaining in these hybrids are considered among the strongest evidences in support of Boveri's hypothesis that the individual chromosomes persist and do not mix in the resting stages of the nuclei.

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EXPLANATION OF PLATES.

PLATES I TO IV.

ABBREVIATIONS.

bl'po., Blastopore.
cd., Chorda.
cc'drm., Ectoderm.
emb., Embryo.
en'drm., Endoderm.
lns., Lens.
ms'drm., Mesoderm.
pi'bl., Periblast.
ylk., Yolk.

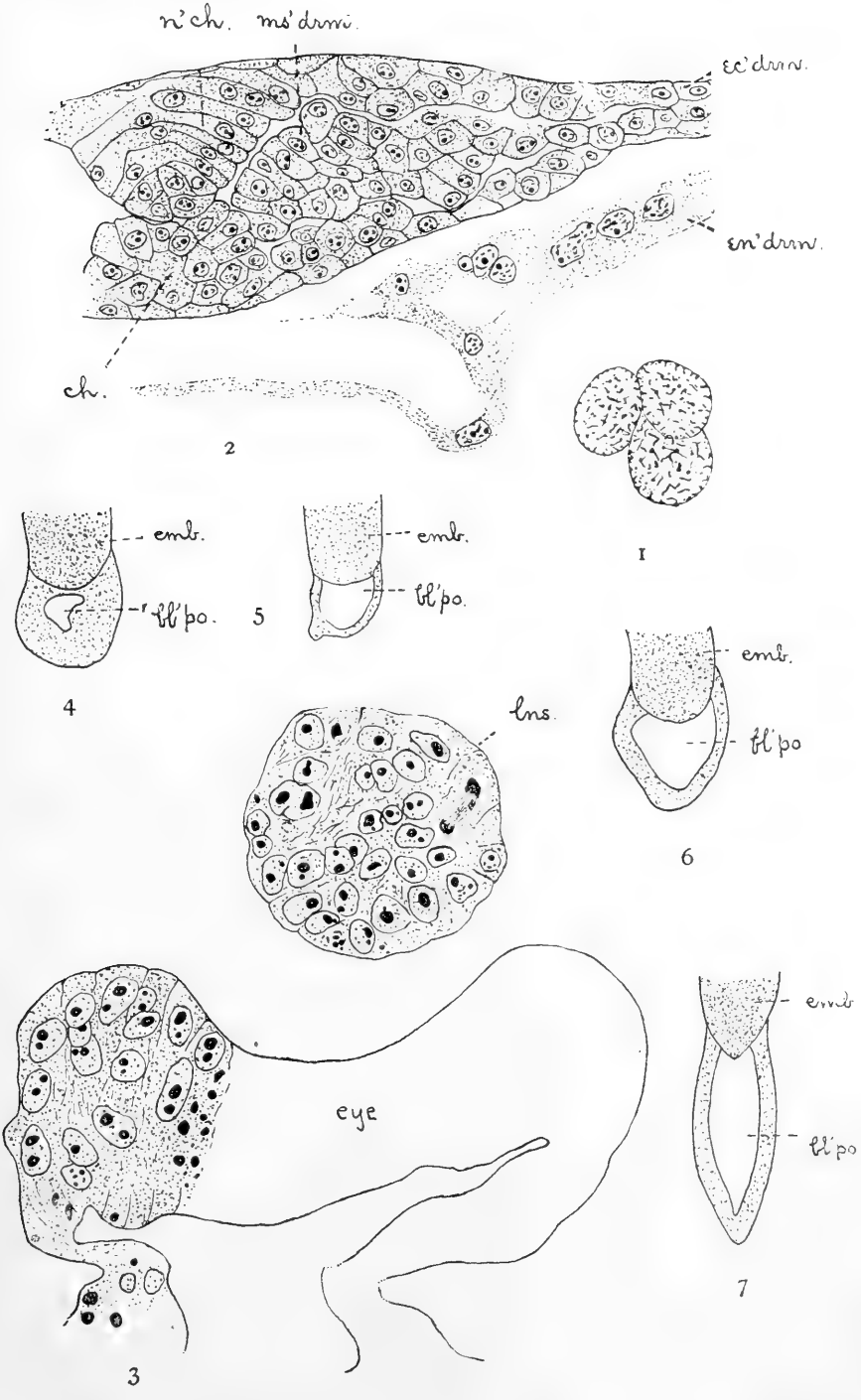
PLATE I.

FIG. 1. Conjugation of pronuclei in a dispermic Fundulus hybrid egg.

FIG. 2. Cross section of a Fundulus hybrid embryo that was nearing the closure of the "blastopore." It shows the ectoderm, endoderm, mesoderm, neural cord, chorda and periblast.

FIG. 3. Horizontal section through the eye of a Fundulus hybrid embryo that had come to the close of its development. Very few of the cell boundries can be made out. Cells of the cup are arranged in more or less distinct rows. Nucleoli numerous and large. One cell in the lens in the process of division.

FIGS. 4, 5, 6 and 7. Caudal end of four Fundulus hybrid embryos which had come to the close of their development. Shows four types of abnormal "blastopore" closure.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eye
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PLATE II.

FIGS. 8, 9 and 10. Types of abnormal "blastopore" closure in the Menidia hybrids.

FIG. 11. Late anaphase of the first cleavage of a normal Fundulus heteroclitus egg. All of the chromosomes are of the long type.

FIG. 12. Anaphase of the first cleavage of a normal Menidia notata egg. All of the chromosomes are of the short type.

FIG. 13. Chromosomes of Fundulus heteroclitus and Menidia notata drawn to the same scale. Both are taken from the spindle shown in Figure 14.

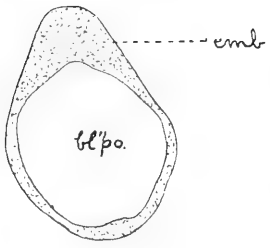
FIG. 14. Anaphase of the first cleavage of a Fundulus hybrid egg. To the right in each half of the spindle occur only the short chromosomes; to the left, only the long ones. ♂ Menidia. ♀ Fundulus.

FIG. 15. Anaphase of the first cleavage of a Menidia hybrid. To the right are all long chromosomes; to the left, all short ones. ♂ Fundulus. ♀ Menidia.

FIG. 16. Metaphase of first cleavage of Fundulus hybrid. The large chromosomes to the right. ♂ Menidia. ♀ Fundulus.

FIG. 17. Metaphase of the second cleavage of a Menidia hybrid. The long chromosomes to the right. ♂ Fundulus. ♀ Menidia.

FIG. 18. Half of an anaphase spindle of the second cleavage of a Menidia hybrid. All of the long chromosomes are to the left. ♂ Fundulus. ♀ Menidia.



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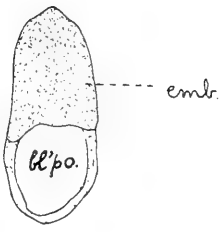

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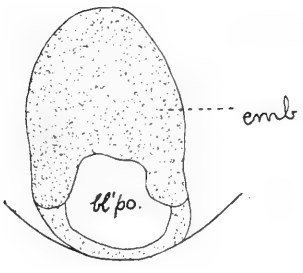

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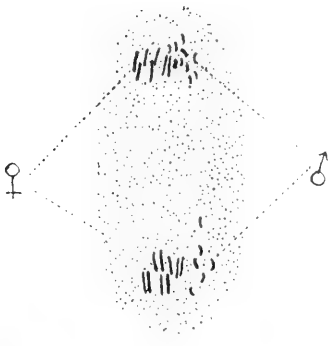

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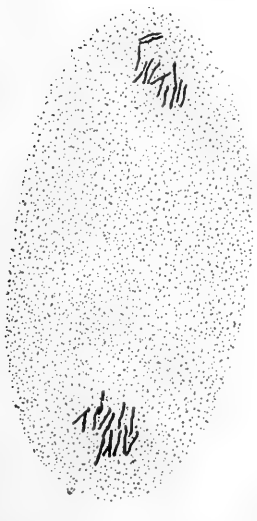

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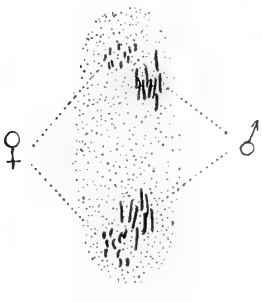

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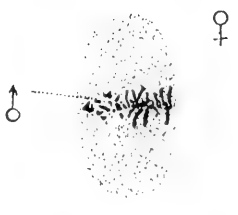

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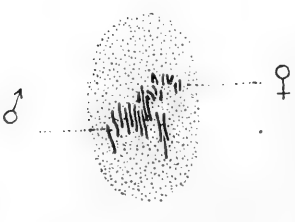

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PLATE III.

FIG. 19. Anaphase of the third cleavage of a Menidia hybrid. In the upper half of the spindle the two kinds are clearly bilaterally distributed. ♂ Fundulus. ♀ Menidia.

FIG. 20. Early anaphase of the fourth cleavage of a Menidia hybrid. The two kinds of chromosomes are evidently mingled. ♂ Fundulus. ♀ Menidia.

FIG. 21. Chromosomes from a fourth cleavage spindle of a Fundulus hybrid. The cell was in anaphase. The chromosomes of the drawing are such of one end of a spindle in a single section as could be distinctly made out. They are drawn by only the partial aid of the camera. Each chromosome is faithfully reproduced so far as its form and size are concerned but its position with reference to its neighbor is not in every instance retained in the drawing. ♂ Menidia. ♀ Fundulus.

FIG. 22. Anaphase of cell from middle cleavage of a Menidia hybrid. The two kinds of chromosomes are mingled. The short ones, as a whole, are nearer the pole than the long ones, so that they form a band extending across the spindle. ♂ Fundulus. ♀ Menidia.

FIG. 23. Telophase of the third cleavage of a Menidia hybrid. Only one-half of the spindle is shown. Of the two groups of vesicles the smaller ones, nearer the poles, are probably from the short chromosomes. The group of larger vesicles is composed for the most part of the long chromosomes not yet so far along in their metamorphosis. ♂ Fundulus. ♀ Menidia.

FIG. 24. Late anaphase of a cell from middle cleavage of a normal Menidia notata. Only the small type of chromosomes are present. Compare with corresponding stages in Figures 22, 26 and 32.

FIG. 25. Early anaphase of cell from early cleavage of a normal Fundulus heteroclitus egg. Only the long chromosomes are present.

FIG. 26. Late anaphase of a cell from later cleavage of a normal Fundulus heteroclitus egg. Only the long type of chromosomes are present. Compare with corresponding stages shown in Figures 22, 24 and 33.

FIG. 27. Cells from middle cleavage of a Menidia hybrid. They show the double nuclei in these cells.

Fig. 28 is omitted on purpose.

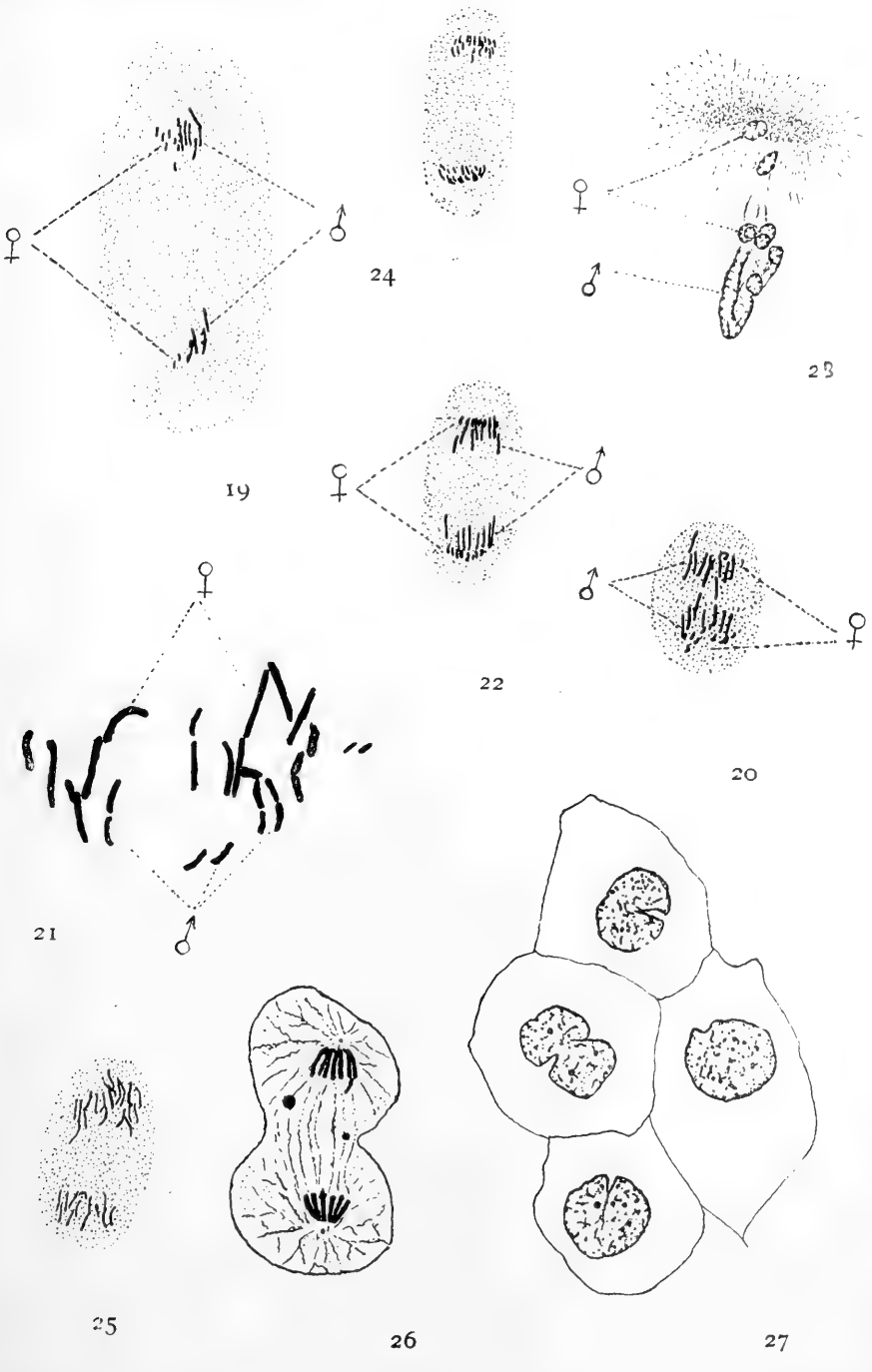
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PLATE IV.

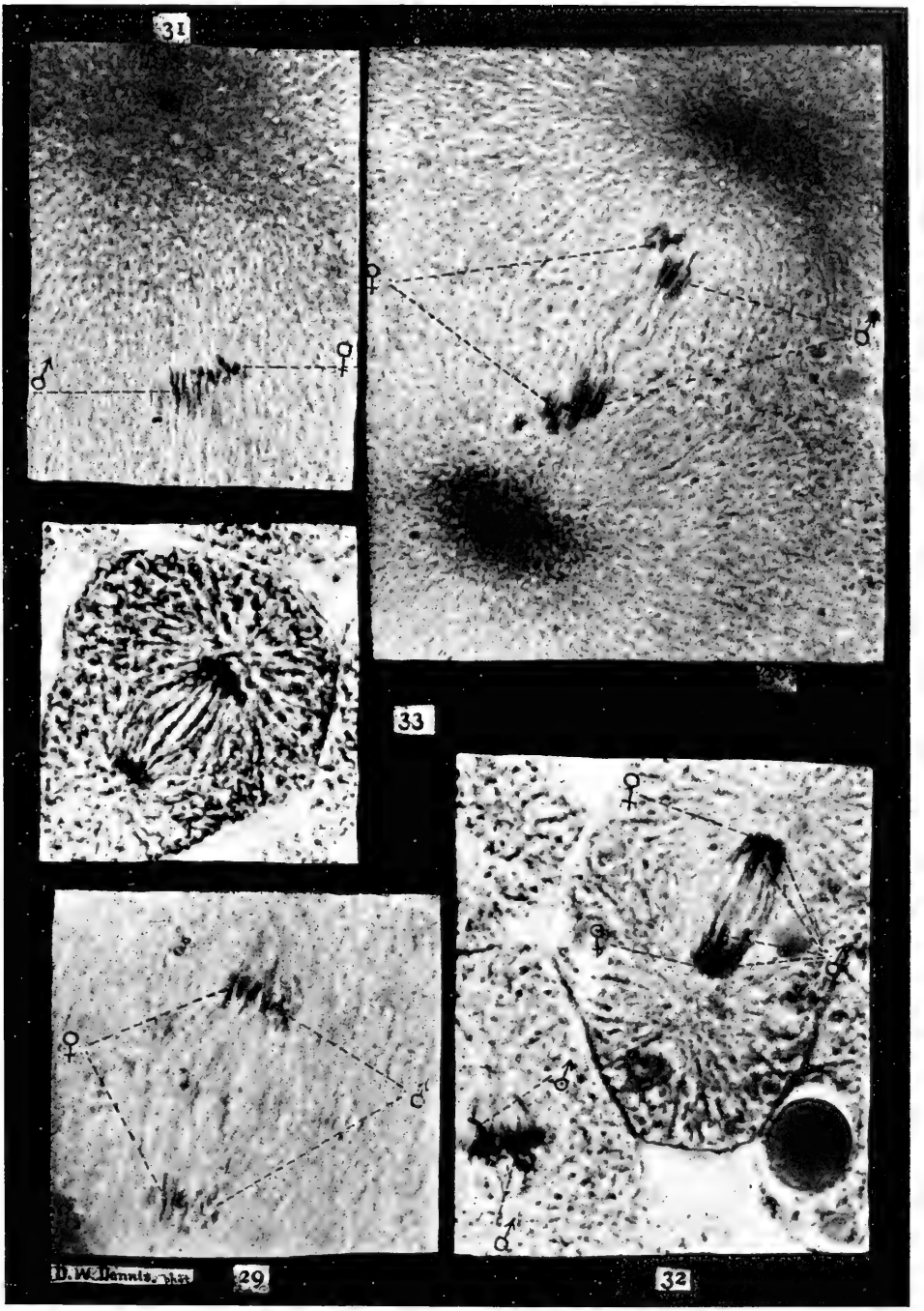
Fig. 29. Anaphase of the first cleavage of a Fundulus hybrid egg. The small Menidia chromosomes introduced by the sperm are grouped to the right.

Fig. 30. Anaphase of the first cleavage of a Menidia hybrid egg. Here the long Fundulus chromosomes have been introduced by the sperm and are grouped on the right in the spindle.

Fig. 31. Half of an anaphase spindle of the second cleavage of a Menidia hybrid. The short and the long chromosomes are grouped to the right and left respectively. ♂ Fundulus. ♀ Menidia.

Fig. 32. Late metaphase and late anaphase of cells from middle cleavage of a Menidia hybrid egg. In the latter the long chromosomes extend for a considerable distance along the spindle fibres while the short ones are nearer the poles and form a band across the spindle. ♂ Fundulus. ♀ Menidia.

Fig. 33. Late anaphase of a cell from middle cleavage of a Menidia notata egg. All the chromosomes are short. There are no long straggling chromosomes as in the hybrid cells (Fig. 32.) See also Figures 22 and 26.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ON THE LUNG OF THE OPOSSUM.

BY

JOHN LEWIS BREMER, M. D.

From the Embryological Laboratory of Harvard Medical School.

WITH 11 TEXT FIGURES.

The lung of the new-born opossum in the pouch shows peculiarities, already partly described by Selenka, which make it appear that respira-

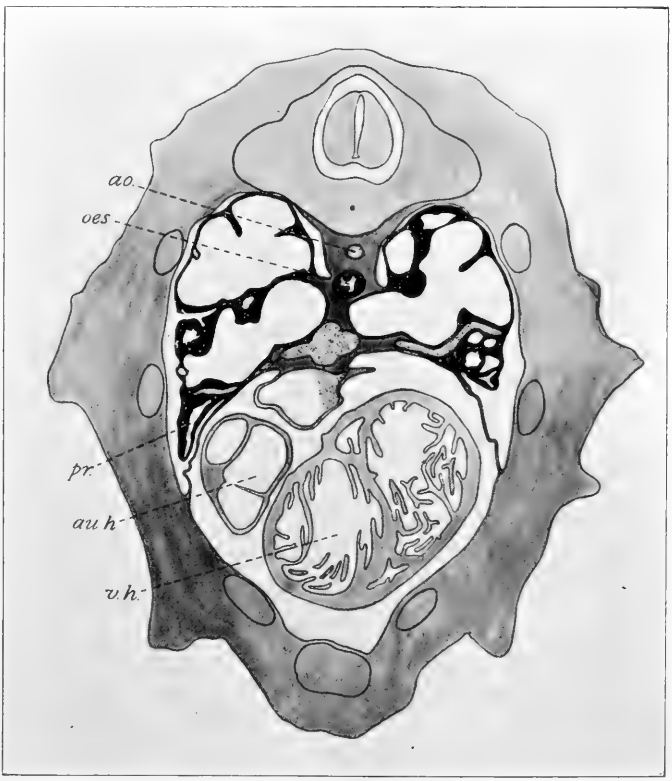

FIG. 1. Opossum, 10.5 mm. trans. Series 614, No. 305. *Ao*, aorta; *oes*, oesophagus; *au*, auricle, *ven*, ventricle of heart; *pr.*, new bronchial bud.

tion is carried on in specially modified bronchi and bronchioles before the infundibular portion of the lungs is developed. Selenka's descrip-

tion, found on p. 159 of his "Entwickelungsgeschichte der Tiere," is as follows:

"The lungs of opossum have to develop into functioning breathing organs within the last three days of uterine life. There is neither the available material nor the necessary time to make a very great number of alveoli and prepare them for breathing (as is completely done in

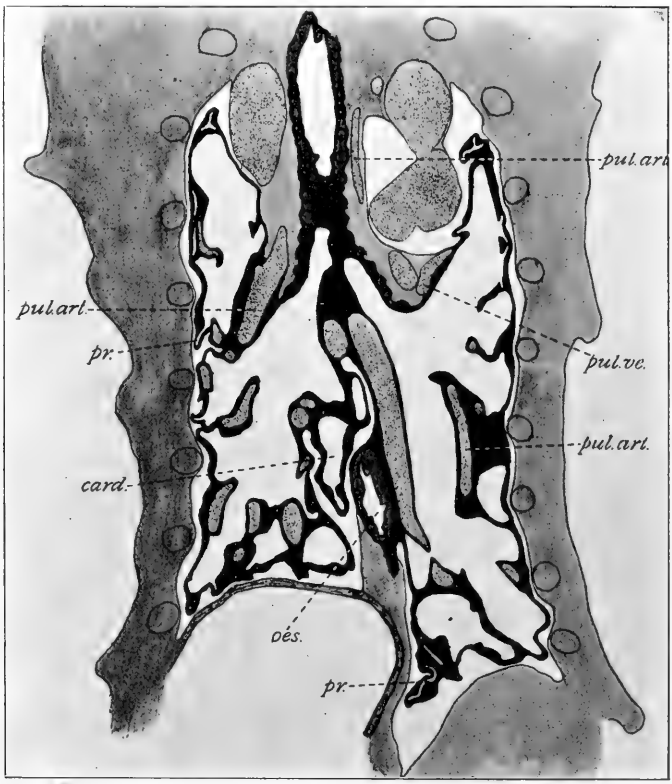

FIG. 2. Opossum, 12.5 mm. frontal. Series 618, No. 338. *Pul. art*, pulmonary artery; *pul. ve*, pulmonary vein; *oes*, oesophagus; *pr*, new bronchial bud; *card*, part of cardiac lobe.

fœtal life in placentalia). Only a few dozen large air-chambers, as a provisional breathing apparatus, can be made, which later, during the life in the pouch, develop by the growth of partitions into a richly branched bronchial tree. The lung may be said to be of rapid growth inasmuch as the alveoli are ready for breathing in a remarkably short time; but its growth is slow if we consider the increase in the number

of alveoli as a gauge. Probably in this wonderful development of the opossum lung, the forces at work in evolution are reproduced, for the lung of a new-born opossum has exactly the form of a reptilian lung."

In the main this description is correct, but it seems to me imperfect in some respects. The opossums examined by me were: first, six newborn, taken from the same pouch, ranging in size from 10.5 to 12.5 mm.; second, two of about 14 cm.; third, two young adults and one old adult.

The lungs of the smallest opossums correspond to the description of Selenka, as they are composed of a few large air-chambers, opening almost directly into the main bronchus, which is itself an elongated chamber. The appearance in section is shown in Figs. 1 and 2, a trans-

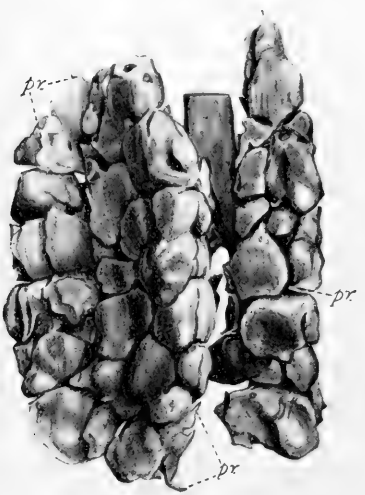

Fig. 3.

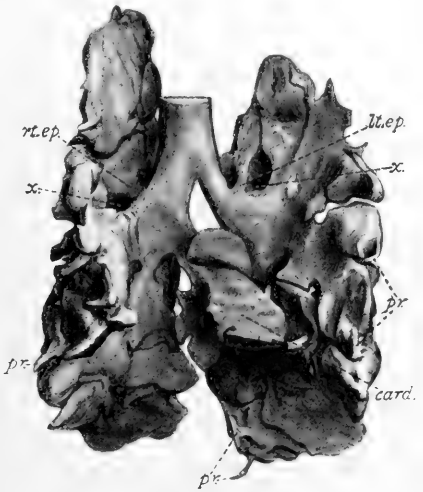

Fig. 4.

Fig. 3. Cast of lung of 12.5 mm. opossum, seen from behind and from the left. *pr*, new bronchial buds.

Fig. 4. Cast of lung of 12.5 mm. opossum, seen from the front and a little above. *pr*, new bronchial buds; *card*, cardiac lobe; *lt. ep*, *rt. ep*, left and right eparterial bronchi; *x*, *x*, groove for pulmonary artery.

verse and a frontal section of a 10.5 mm. and a 12.5 mm. opossum respectively; and the general form is shown in Figs. 3 and 4, drawings of a cast of the lung of the 12.5 mm. opossum obtained by Born's wax-plate method. Selenka is wrong, however, in speaking of alveoli; the large chambers correspond to bronchi and bronchioles; infundibula and alveoli are lacking.

In the lungs of placentalia growth in the embryo is accomplished by the branching of the small tubes of cuboidal cells and with narrow lumen, which represent the bronchi; each new limb in turn sends out

new buds, all of which are like the parent stem, pushing into the surrounding mesenchyma. Just before birth a new kind of bud, with a different system of division, is developed from the end of the last set of branches, and these form the infundibula and alveoli, the true breathing portion of the lung. In the young opossum, which is transferred to the pouch when only about 10 mm. long, breathing must be carried on at the same time as the growth and branching of the bronchial tree; so instead of the usual short buds of cuboidal epithelium, as found in placentalia, in the young opossum large chambers are found, representing the narrow tubes, but lined with peculiar epithelium so that they may serve as respiratory organs. These chambers are not, however, to be considered alveoli, but bronchi and bronchioles: and they retain their power of sending off new buds or branches, which may be seen in the model and in the sections as hornlike processes, hollow and slender at first (soon widening into large chambers), pushing into the surrounding mesenchyma, and giving evidence that Selenka's idea of division into a bronchial tree by means of newly forming partition walls is wrong. The lung of the newborn opossum is composed of a simple system of branching bronchi and bronchioles, dilated and lined with modified epithelium to allow for breathing, but retaining their power of further growth.

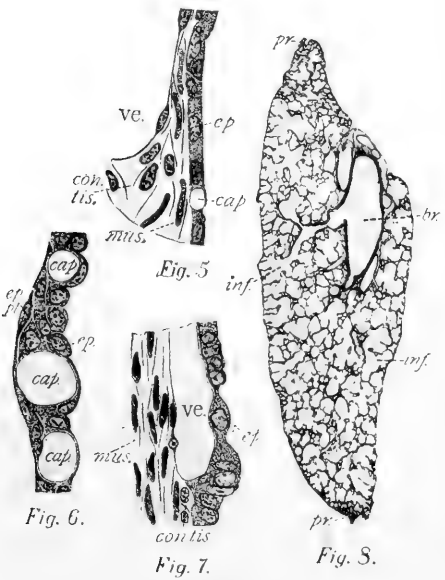

FIG. 5. Opossum, 12.5 mm. Series 618, No. 303. *Ep*, epithelium lining air-chamber; *mus*, muscle; *con. tis*, connective tissue; ve, vein; *cap*, capillary.
FIG. 6. Same as Fig. 5. *Ep. pl*, epithelium of pleura.
FIG. 7. Lacerta. Series 604, No. 325.
FIG. 8. Opossum, 14 cm. Trans. section of lung. *Pr*, new bronchial branches; *inf*, infundibula.

On examining the epithelium lining these air-chambers, we find what seems to me a transitional stage between cuboidal and "breathing epithelium" (see Figs. 5 and 6). Directly over the capillaries, three of which are cut across in Fig. 6, the cells have become squamous, with a thin plate and the nucleus lying between the blood vessels; but the plates are not to be compared in thinness with those of the human lung, for instance (in one cell in Fig. 6 the nucleus lies in the plate); and the meshes of the capillaries are so

wide that many cells remain cuboidal, having no capillary over which to spread a plate. This peculiar epithelium extends not only over the inner surface of all the air-chambers, but also over the main bronchi as far up as the beginning of the rings of cartilage.

If we compare these lungs with those of some reptiles we find that they are similar both in the arrangement of air-chambers opening into a dilated main bronchus, and in the character of the epithelium, as is shown in Fig. 7, drawn from the lung of Lacerta. Also in both the opossum lungs and reptilian lungs there are bands of muscle fibres running circularly around the central air-chamber or bronchus, whose probable function is to contract the lung and force the air out during expiration. Still further, in the reptilian lung the arrangement of the bronchial branches is symmetrical, both right and left bronchus being provided with one branch anterior to, and another posterior to the pulmonary artery; and if we examine the drawing (Fig 4) and the diagram (Fig. 10) made from the same opossum, we find that in the new-born opossum also there is one bronchial branch in front of and another behind the artery in both right and left lung. This was found in five out of the six new-born opossums; one was rendered useless for serial work. In other words, the new-born opossum has an eparterial bronchus on both right and left sides; that on the left is always the smaller and slightly lower placed, and the air-chambers supplied by it do not form the apex of the lung; still in spite of its small size and relatively low position, it is distinctly above the first ventral bronchus and behind the artery and so corresponds to the eparterial bronchus of the right lung, and may be considered as making the two lungs symmetrical and reptilian in type, as no placental mammalian lungs are. This symmetry is marred by the presence of a large cardiac lobe on the right side, of which I can find no trace on the left. Still, as regards general appearance, character of the lining epithelium, and symmetry of bronchial branches (with this one exception), these lungs are, as Selenka says, reptilian.

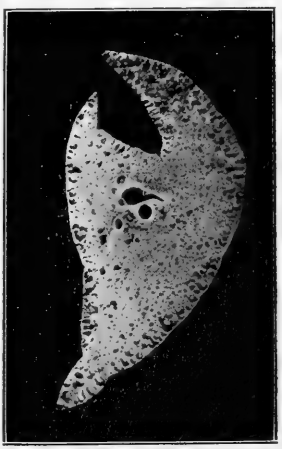

Fig. 9. Photograph of cut surface of lung of young adult opossum.

Let us now trace the growth of this lung further. On looking at Fig. 8, a section of the lung of a 14 cm. opossum, we find the primary bronchi and their early branches now provided with a thick coat, partly due to the multiplication of the circular muscle fibres already mentioned, while

the lining epithelium has reverted to the cuboidal type or even become cylindrical. We have found now the reason for the peculiar epithelium seen when these passages were breathing spaces; it was a compromise allowing enough oxygenation of the blood for an animal whose existence is passed in the mother's pouch, and yet not far enough removed from the cuboidal type to make it hard to revert to it.

The bronchial tree has become quite complicated and at the surface of the lung may be seen in cross-section new hornlike processes (pr.) representing newly formed branches. But with these are terminal pieces of much larger size, often with triple branching, seen chiefly on the surfaces of the lung where growth has nearly ceased. They represent a new element in the opossum lung, but one found in placental lungs just before birth, namely the infundibular portion of the lung (inf.). They may be seen forming a cortex in Fig. 9, a photograph of the cut surface of the lung of a young adult opossum; but with age they become inconspicuous because more evenly distributed. They mark the end of the stage of rapid growth, for from these infundibula no branches, unless we count the alveoli, are given off; and so they are absent from all activity growing portions (such as the borders in Fig. 8), their places being taken by the hornlike processes, which are capable of further growth. The lung has changed from a reptilian to a mammalian type partly by the multiplication of the bronchial branches, but chiefly by the addition of a new class of air chambers, growing from the ends of the bronchioles, but differing from them in that the spaces are dilated instead of horn-shaped or tapering, and are lined with true "breathing epithelium," with narrow-meshed blood vessels and very thin plates.

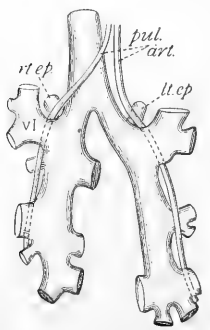

FIG. 10. Diagram, lung of opossum of 12.5 mm. seen from in front. *Pul. art.*, pulmonary artery; *card.*, cardiac lobe; *lt. ep*, *rt. ep.* left and right eparterial bronchi. VI, first ventral bronchial branch.

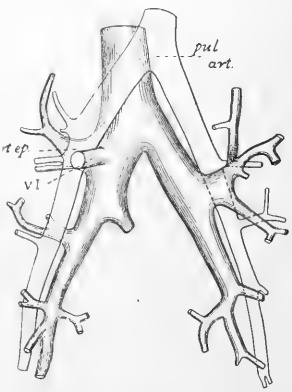

FIG. 11. Diagram: Lung of opossum of 14 cm. seen from in front.

The opossum lung changes from reptilian to mammalian also in the loss of the left eparterial bronchus. How this comes about I am unable to state for the lack of the necessary stages, for already in the opossum of 14 cm. the change is complete, as can be seen in Fig. 11, a diagram of the bronchial branches and the arteries of an opossum of that size, where no trace of a left eparterial bronchus remains.

Following Selenka's suggestion, then, it seems to me that we find in the lung of the opossum an epitome of the evolution from reptilian to mammalian lung, and that the chief points are the loss of the left eparterial bronchus in mammals, and the addition to the reptilian lung, which consists only of bronchi and bronchioles, of a new apparatus, with a different and more complicated system of branching, and with walls better adapted for breathing—the infundibular portion of the lung.

ENAMEL IN THE TEETH OF AN EMBRYO EDENTATE (DASYPUS NOVEMCINCTUS LINN).[1]

BY

A. M. SPURGIN, M. D.

WITH 2 PLATES.

Tomes was the first to work on the embryology of the teeth of the nine-banded armadillo (*Dasypus novemcinctus* L.). In 1874, he examined two embryos, one early and one relatively late. The exact length of these embryos I have been unable to ascertain, but in the early one a layer of dentine had been deposited. He said that the stellate reticulum or enamel pulp was absent, and that he failed to find any enamel or anything like it upon the teeth. He regarded the enamel organ as rudimentary, stating that an enamel organ was present in all tooth-germs, and that it was entirely independent of any subsequent development of enamel.

In 1884, Pouchet and Chabry examined embryos of *Orycteropus capensis,* and *Bradypus tridactylus.* In an embryo of the former, of 32 cm., they found a typical rudimentary incisor with an enamel organ and dental papilla in which a layer of dentine had been deposited. An embryo of 12 cm. of *Bradypus tridactylus* showed an enamel organ covering the dental papilla in which a layer of dentine had appeared. In an embryo of the same animal of 23 cm. in which the teeth had erupted, they described the dentine, vasodentine, and outer coat of cement of the typical adult tooth. They found no enamel, and state that the stellate reticulum was absent in the enamel organ of the sloths.

As early as 1828, A. Brants found a rudimentary incisor in the lower jaw of *Bradypus tridactylus.* P. Gervais in 1873 confirmed this discovery. Burmeister made a similar discovery in the fossil *Scelidotherium leptocephalum.* Flower, in 1869, described a rudimentary incisor in the lower jaw of *Tatusia Peba* (*Dasypus novemcinctus*), and in 1877 Reinhardt observed as many as four in the lower jaw of the same animal.

Hensel has shown that the armadillos, *Dasypus novemcinctus* and *D. hybridus* Desm. are diphyodont. In an examination of thirty five skulls

[1] Contributions from the Zoölogical Laboratory of the University of Texas. No. 51.

of the former, he found some with milk teeth and some showing the change to the premanent set. The last tooth had no predecessor, and the teeth were not changed until the animal had nearly reached the adult stage. On examination of two skulls of *Dasypus hybridus* a similar condition was found. A rudimentary incisor was also found in this animal. The milk teeth have been described as two-rooted, but Tomes holds that this appearance is due to the absorption set up by the pressure of the succeeding permanent teeth.

In 1889, O. Thomas found in two young specimens of Orycteropus of fourteen and eighteen inches respectively, a complete though rudimentary set of milk teeth in each jaw, none of which were in the premaxillæ. They were all minute, and this fact led him to think it very doubtful that they would ever have cut the gum. Unfortunately his material was limited, and he made no histological investigation, so we know nothing of the structure of the enamel organ at this stage. Thomas has also examined specimens of Bradypus, Cholœpus, and Dasypus, apparently of a suitable age, and could find no trace of a milk dentition; he says, however, that the possibility still remains that in younger stages uncalcified tooth-buds of such teeth may be present. Tomes and Flower have also examined fœtal Cholœpus and Bradypus and have found no trace of any milk dentition.

In 1892, Röse (92[a], p. 507) observed in an embryo of *Myrmecophaga didactyla* 20 cm. long, at the point of the jaw, where in other cases the tooth-buds are connected with the mouth epithelium, a row of exceptionally high papillæ. He thinks that very probably in younger stages the tooth-buds were formed at this place but were not further developed. In the same year, in an embryo of 7.6 cm. of *Manis tricuspis* and one of *M. javanica* of 9 cm., Röse[2] found well defined dental folds in both upper and lower jaws, and in the lower jaws rudimentary club-shaped tooth-buds. He has shown by examination of older specimens that they subsequently disappear.

The only work that has been done on the teeth of the armadillo since that of Tomes has been done by Röse and by Ballowitz who worked at the same time but independently of each other.

In 1892, Röse examined two embryos, one of *Dasypus novemcinctus* of 7 cm. and one of *D. hybridus* of 6 cm. In the first-named embryo he found an enamel organ composed of an inner and an outer epithelial layer and a well developed stellate reticulum. He did not describe any

[2] The mounted slides of these embryos had been furnished by Max Weber ('91) who had not found any indication of the dental folds.

stratum intermedium. He described the buds for the permanent teeth as arising from the outer layer of the enamel organ, and not, as Tomes has shown it in one of his figures, as coming from the mucous membrane of the mouth cavity. The bud for the eighth tooth, which has no predecessor, he described and illustrated as arising directly from the mucous membrane of the mouth cavity. With the exception of the first, the teeth are bicuspid. The tooth-buds of two rudimentary incisors were described but no dentine had been deposited. Röse found the same general condition in the embryo of *Dasypus hybridus*. Besides two rudimentary incisors, there were seven back teeth, the first two having single cusps. On the whole the development was further advanced than in the other embryo, dentine having been deposited in the rudimentary incisors as well as in some of the back teeth. Röse found in connection with the enamel organ of both of these rudimentary incisors, secondary buds coming from the outer epithelial layer, the one from the second incisor being best developed. He remarks that this does not cut off the possibility that this tooth may also have a successor in the later change of teeth. He states that while the embryos examined by him had no enamel, they did have, as a secretion product of the enamel cells, a thin structureless membrane lying directly against the dentine and exactly corresponding to the formation which in other animals we call Nasymth's membrane.

Ballowitz examined two embryos of *Dasypus novemcinctus* of 6 and 8 cm. respectively. He found a typical enamel organ, with inner and outer epithelial layers, stratum intermedium, and well developed stellate reticulum. He describes the processes of the inner columnar epithelial cells, generally known as Tomes' processes, but says he has not been able to explain them. He states that very soon after the first layers of dentine have been deposited, the outer layer of cells disappears and the stellate reticulum is replaced by connective tissue. He says that while it is true that the inner epithelial layer and stratum intermedium remain over the calcified dentine in an unbroken layer, they have undergone a considerable change; the inner layer loses its columnar shape and becomes flattened, the stratum intermedium is reduced, so that only two or three layers of flat cells can be found on the cusps. Whether these cells have anything to do with the development of Nasymth's membrane, or whether in these teeth such a membrane was present at all, Ballowitz says, it was impossible to decide. In the tooth-buds of the larger embryo, which were separated only by connective tissue, he found secondary buds coming off from the lingual side of the outer epithelial layer of the enamel organ. No rudimentary incisors were described, and I presume none

were observed. The point Ballowitz lays most stress upon is the finding of an epithelial ring at the base of the dental papilla which is a portion of the enamel organ constricted off from the lower edge of that organ. He has shown this epithelial ring to persist in the adult, and he regards it as essential to the development of the dentine in these continuously growing teeth. He quotes from A. von Brunn's work on the enamel organ in support of this theory, but I have been unable to see this article. Ballowitz denies that the presence of the stellate reticulum and stratum intermedium have any close connection with the deposition of enamel, stating positively that at no time can enamel be deposited in the *Dasypus novemcinctus,* and that the only functions of the enamel organ are: to give form to the developing tooth, to stimulate the odontoblasts to deposit dentine, and to give off the epithelial ring which is necessary to the continued development of the dentine.

A year ago, Dr. W. M. Wheeler, of the School of Zoology, had the good fortune to secure four embryos of the *Dasypus novemcinctus* from an adult female which had been kept in the laboratory for several weeks. The embryos were removed immediately after the animal had been chloroformed, and were hardened for six weeks in Müller's fluid, primarily for studying the placentation. He found four placentæ inclosed in one amnion (Plate I), but has not since had the time to study the subject further. Dr. Wheeler very kindly furnished me the material for working on the embryology of the teeth, but owing to the pressure of other work, nothing was done until this year.

The largest embryo of 9 cm.[3] and one measuring 8.5 cm. were selected. From the larger embryo, longitudinal sections of the lower jaw were made, and by making a sagittal section of the upper jaw, both longitudinal and transverse sections were obtained. They were imbedded in celloidin, cut 25 micra thick, and stained in hæmatoxylin and eosin, but were not kept in series. From an embryo of 8.5 cm. both longitudinal and transverse sections of the lower jaw and longitudinal sections of the upper jaw were made. They were imbedded in paraffin, cut 10 micra thick, mounted in series, and stained with iron hæmatoxylin.

In the longitudinal sections of the lower jaw of the 8.5 cm. embryo, I found five rudimentary incisors and eight back teeth. The jaw measured 11 mm. from the tip to the posterior edge of the last tooth-bud. The first incisor was found 1.8 mm. from the tip, the width of

[3] In all cases the measurements given are from the crown of the head to the base of the tail.

the jaw at this point being 1.5 mm. The incisors were separated from each other by about .5 mm. These rudimentary incisors diminished in size and degree of development from behind forward as shown in Plate II, Fig. 1. They were separated by connective tissue with the exception of the last two, which were separated by a rather large piece of cartilage. A similar piece of cartilage behind the last tooth and a somewhat smaller piece growing up between the third and fourth teeth (Plate II, Fig. I), would seem to indicate that sockets were to be formed for at least the last two. The shape of the first three teeth is that of a true incisor with a single cutting edge, while the shape of the last two is nearly that of a typical cuspid with a single somewhat prominent cusp.

On each of the rudimentary incisors a layer of enamel has been deposited. The relative thickness, which diminishes from the back tooth forward, is represented in Plate II, Fig. 1, by the black line. Under the low power, the enamel appears as a dark band which, in many sections, has been pulled away from the dentine and fractured in the direction of the enamel rods. This was due to the sectioning, since the tissue had not been completely decalcified by the Müller's fluid. Plate II, Fig. 2, shows this condition in a high power drawing of one of the incisors. With the $\frac{1}{12}$-inch oil immersion lens the direction and structure of the enamel rods could be made out. In the fourth and fifth incisors the inner layer of the enamel organ had lost its columnar character; the stellate reticulum had disappeared, and only a few layers of flattened epithelial cells remained over the enamel layer. In the first three teeth, in which the enamel was not so thick, more of the enamel organ remained, and at places away from the central area of the cusp, the columnar cells of the inner layer could be seen. The cells in the immediate area of the cusps were flattened as in the case of the last two teeth. This clearly indicates that the enamel in the last two teeth has been completely laid down, while more may yet be deposited from the columnar cells in the three anterior teeth. No Nasmyth's membrane could be found, and no secondary buds were observed in any of the rudimentary incisors. These buds were not to be expected, since the development had advanced considerably further than in the 6 cm. embryo of *Dasypus hybridus*, in which Röse demonstrated their occurrence. Although I carefully examined the sections from the upper jaws of both embryos, I failed to find any trace of buds for rudimentary incisors.

From a study of the longitudinal and transverse sections from the lower jaw of the 8.5 cm. embryo, it could be seen that the tooth-buds of the eight back teeth were almost completely surrounded by cartilage. The two plates of cartilage forming the groove in which the teeth were

developing sent prolongations between them which roughly followed the contour of the teeth. The tooth-buds, however, were close together and complete septa had not as yet been formed between them. In all the back teeth except the eighth, a thin layer of dentine had been deposited and in a few of them it was calcified. On the whole the development of the teeth in the lower jaw was in advance of that of the upper. In the embryo of 9 cm. the development was still further advanced, and calcified dentine was found in most of the teeth. Plate II, Fig. 3, shows the first back tooth with a well developed layer of enamel appearing under the low power as a much darker band than the dentine, and broken at frequent intervals in the direction of the enamel rods in the process of sectioning. As in the first three rudimentary teeth, the columnar cells of the inner layer of the enamel organ have become flattened over the thicker portion of the enamel layer, while they still retain their shape over the thinner portions (Plate II, Figs. 2 and 3, ce). The stellate reticulum and outer layer of the enamel organ were still present over the sides of the dental papilla as shown in Fig. 3. The portion marked eo, which has been torn in sectioning from the body of the enamel organ, shows the epithelial ring (er) in process of being constricted off. This has been described in full by Ballowitz and has also been observed by Pouchet and Chabry in the embryo of the sloths. I found this portion of the enamel organ in many sections of both rudimentary and back teeth. In some sections it has been separated from the enamel organ. Plate II, Fig. 4, shows a high power drawing of the enamel and dentine from the same section as Plate II, Fig. 3. The uncalcified dentine is easily distinguished from the darker calcified dentine, being cut off from the latter by a sharp line of demarcation, a condition which was not found in any of the rudimentary teeth. This may indicate that in these teeth the dentine has been completely deposited. If such proves to be the case in later embryos, Ballowitz's theory concerning the epithelial ring would have no weight.

In the fifth and sixth tooth-buds, in the longitudinal sections of the smaller embryo, the buds for the permanent teeth could be seen coming off from the outer epithelial layer of the enamel organ. Plate II, Fig. 5, which shows this, shows also a portion of the enamel organ with inner and outer epithelium, stratum intermedium, stellate reticulum, and Tomes' processes. A thin layer of uncalcified dentine has been deposited. I do not find that the outer layer of the enamel organ is broken through until after the enamel has begun to be laid down. Röse and Ballowitz both describe the breaking up of this layer as taking place shortly after

a thin layer of dentine has been deposited and much earlier than is the case with most animals. Röse (93, p. 448) describes the same condition in the teeth of reptiles. The condition I find is exactly what we should expect. As is well known, the dentine is deposited first and the outer layer and stellate reticulum of the enamel organ do not disappear until after the first layers of enamel have been deposited (Sudduth, 86, p. 640). Röse and Ballowitz, however, found no enamel and Ballowitz describes the early degeneration of the entire enamel organ.

The bud for the last tooth, which has no predecessor in the milk-dentition, was considerably smaller than the other seven. A well-rounded dental papilla was present, and the enamel organ was connected with the enamel organ of the seventh tooth by an epithelial band consisting of several layers of cells (Plate II, Fig. 6). I could trace this band distinctly through ten or twelve sections from the longitudinal series of both upper and lower jaws of the 8.5 cm. embryo. I was also able to follow it in the transverse serial sections of the lower jaw of the same embryo.

As will be seen, the results of my work on the tooth embryology of the armadillo differ in several important points from those of Röse and of Ballowitz. Röse described and figured the bud for the last tooth as coming from the mouth cavity direct, but it had not as yet expanded into the enamel organ and no dental papilla was present. What Röse had was probably a tubule of one of the glands which appear in the region behind the last tooth-bud.

As has been mentioned, Röse describes as the secretion product of the enamel cells, a thin structureless membrane which lies directly against the dentine and corresponds to the Nasmyth's membrane of other animals. It is very evident that such a membrane does not exist between the dentine and the enamel which I have shown to be deposited later. Röse may have seen a very thin layer of enamel.

I shall not enter into a discussion of the epithelial ring upon which Ballowitz lays so much stress, since I did not have access to the literature upon the subject, but he is certainly wrong in asserting that the only function of the enamel organ is to give the form to the developing tooth, and to give off the epithelial ring. In regard to the presence of the stellate reticulum, Röse offers no explanation. Ballowitz, while recognizing that the stellate reticulum is found only in tooth-buds in which a layer of enamel is afterwards deposited, denies that it has any connection with the deposition of this substance. It is indeed difficult to see how Ballowitz could have failed to see any significance in Tomes' processes, which he described in connection with the enamel cells. He

says that we could adopt Waldeyer's mechanical theory of the enamel pulp as merely serving to make room for the developing tooth, were it not for the fact that the entire enamel organ disappears so early. But I have shown that this is not the case; the breaking up of the outer epithelial layer and disappearance of the stellate reticulum does not take place any earlier in the armadillo than in other animals. While recognizing the importance of the enamel organ in all animals as directing the growth of the dentine and giving the form to the tooth, I do not believe that the stellate reticulum merely subserves a mechanical function; but I regard the finding of enamel in the armadillo as strengthening the view that the stellate reticulum holds pabulum for the first layers of enamel.

I was unable to see Reinhardt's article, but find through Röse's discussion of it that he describes the rudimentary teeth of *Dasypus novemcinctus* as having closed roots and states that they never cut the gum but are later absorbed. He says, however, that the last tooth is sometimes retained in half-grown animals. I did not find the teeth showing any signs of absorption and, as can be seen from Plate II, Fig. 1, they have open roots which are typical of the persistently growing adult teeth. I believe that the teeth will be erupted and thus lost. I am led to this view by the fact that there are indications of the formation of sockets for the last two teeth, and that the teeth are all fairly well developed. Supporting this view, we know that in the Priodontes the teeth in the anterior portion of the jaw are soon lost and that all traces of the sockets disappear. We also know that in *Dasypus setosus,* and the fossil Chlamydotherium, incisors still function.

While Röse and Ballowitz very correctly state that the discovery of a well developed enamel organ in the armadillos tends to show that they are descended from animals whose teeth are more highly organized, I have shown that enamel is still present on the teeth of the milk dentition, and that the gradual reduction of the enamel, as well as that of the incisor teeth, is still taking place. I believe that older stages of the *Dasypus hybridus,* in which, according to Röse, the enamel organ is equally well developed, will show enamel. The question as to whether or not any enamel is present in the tooth-buds of the premanent teeth, and the question as to how long the enamel remains on the milk teeth are matters for further study. The fact that the enamel organ is well developed in the eighth tooth (Plate II, Fig. 6), which has no predecessor in the milk dentition, would seem to indicate that this permanent tooth would have enamel. I attempted to demonstrate this by making dry sections of back teeth taken from several adult armadillos; but as

I was unable to obtain a young animal, all the teeth at my disposal were more or less worn, and if there had been enamel on the teeth at eruption it had been worn off.

Although no enamel is present on the adult teeth of any of the living Edentates, the fossil forms Progmegatherium and Promylodon, from the infra-Pampean beds of Argentina, have been distinguished by Ameghino from the Megatherium and Mylodon as possessing bands of enamel. Burmeister, 91, however, who has also worked on the fossils of this region, disputes Ameghino's statement. Flower (91, p. 204), states that some Glyptodonts occurring in South American beds of an earlier age than the Pleistocene have enamel bands on the teeth. I consider this fact of great weight in showing a possible connection between the Glyptodonts and the living armadillos through the fossil Chlamydotherium, whose teeth resemble those of the Glyptodonts, but have no enamel.

University of Texas, Austin, Texas, May 15, 1903.

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EXPLANATION OF PLATES I AND II.

REFERENCE LETTERS.

c., cartilage.
ct., connective tissue.
ce., columnar cells over thinner portion of enamel layer.
d., dentine.
dp., dental papilla.
e., enamel.
eo., enamel organ.
er., epithelial ring.
eb., epithelial band.
fep., flattened cells of enamel organ.
iep., inner layer of enamel organ.
mm., mucous membrane.
oep., outer layer of enamel organ.
o., odontoblast cells.
sr., stellate reticulum.
si., stratum intermedium.
sb., secondary bud.
stb., portion of second tooth-bud.
ud., uncalcified dentine.
*, space left by shrinkage of specimens.

All figures (except Plate I) were made with the aid of the Camera Lucida and in the process of reproduction were reduced about one-third.

PLATE I.

Photograph of embryos *Dasypus novemcinctus* L., showing placentation. Reduced about one-third.

PLATE II.

Fig. 1. Longitudinal section, lower jaw 8.5 cm. embryo, showing rudimentary incisors. Leitz obj. 3, Oc. 1.

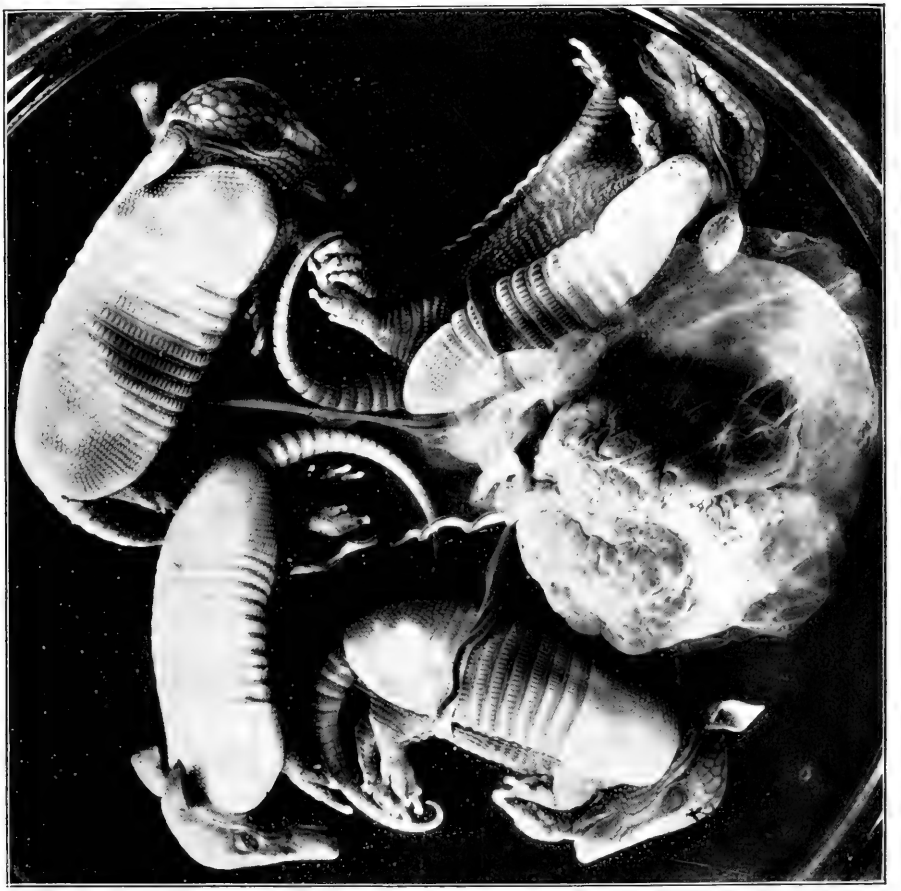
Fig. 2. Rudimentary incisor of Fig. 1 enlarged, showing enamel separated from the dentine and fractured in the direction of the enamel rods. Leitz obj. 7, Oc. 1.

Fig. 3. Longitudinal section, lower jaw 9 cm. embryo, showing enamel in the first back tooth. Leitz obj. 3, Oc. 1.

Fig. 4. Enamel and dentine of Fig. 3 enlarged, showing uncalcified dentine and odontoblast cells. Leitz obj. 7, Oc. 1.

Fig. 5. Longitudinal section, lower jaw 8.5 cm. embryo. A portion of the tooth-bud of the fifth back tooth, showing the secondary bud coming from the outer epithelial layer of the enamel organ. Note Tomes' processes of the inner epithelial layer directed toward the dentine. Leitz obj. 3, Oc. 4, tube drawn out to 20 cm.

Fig. 6. Longitudinal section, lower jaw 8.5 cm. embryo, showing the enamel organ of the eighth tooth-bud still connected by an epithelial band to the enamel organ of the seventh tooth-bud. Leitz obj. 3, Oc. 1.



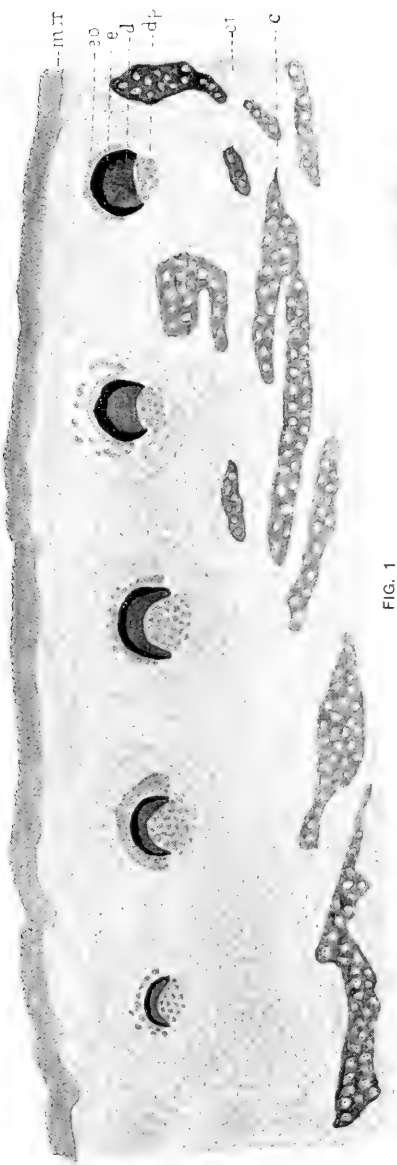


FIG. 1

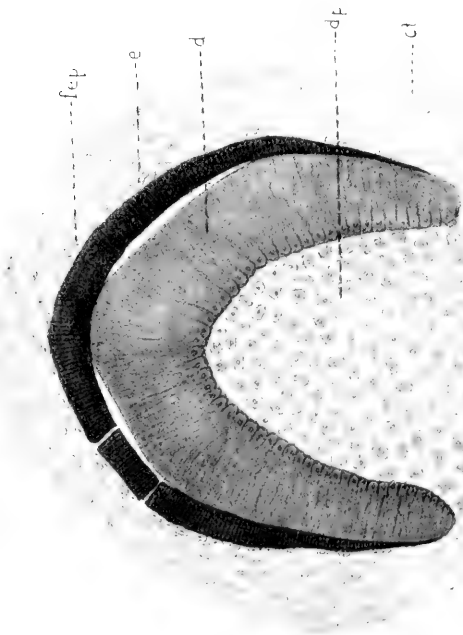


FIG. 2

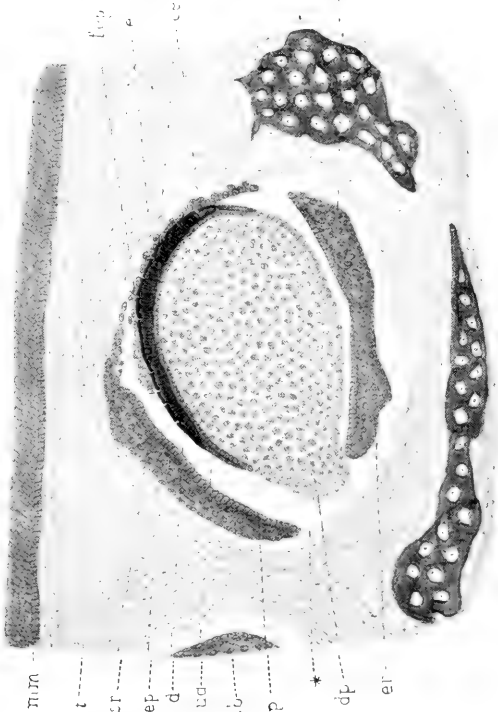


FIG. 3

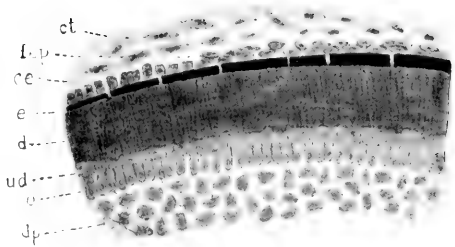


FIG. 4

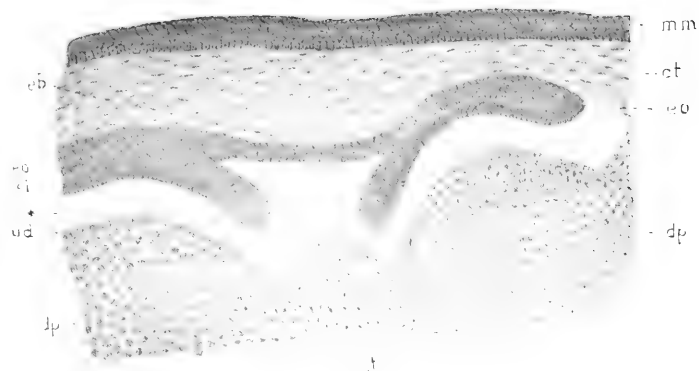


FIG. 6

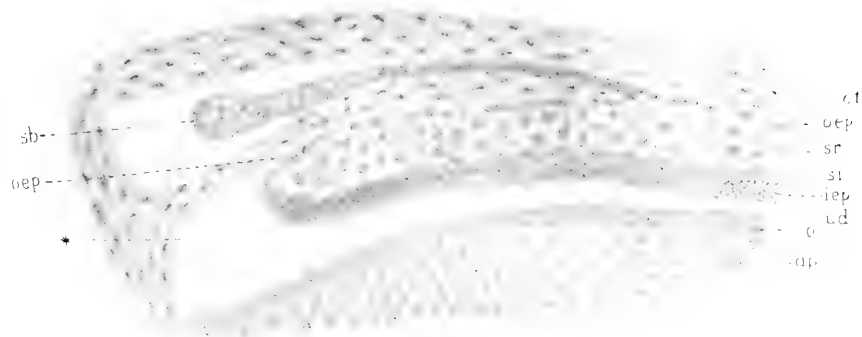


FIG 5

PLATE II

THE EMBRYONIC DEVELOPMENT OF THE OVARY AND TESTIS OF THE MAMMALS.

BY

BENNET MILLS ALLEN.

From the Hull Zoological Laboratory, University of Chicago.

WITH 7 PLATES AND 5 TEXT FIGURES.

This work was carried on with the aim of solving the following problems: (1) The origin and development of the seminiferous tubules and their homologues in the ovary; (2) the origin, development and homologies of the rete tubules together with their relations to the Malpighian corpuscles of the mesonephros on the one hand, and to the seminiferous tubules of the testis on the other; (3) the origin, development and homologies of the connective tissue elements and interstitial cells of the ovary and testis.

Incidental to the solution of these problems, the work has involved to a greater or less extent a consideration of the following allied problems: (1) The development of sex cells; (2) the morphological phases of sex differentiation; (3) cell degeneration in the sex gland and rete; (4) the degeneration of the mesonephros and the development of the Wolffian and Müllerian ducts.

This work, covering as it does a very broad field, naturally touches upon many points that have already been treated by previous workers. Although much has been written upon this subject there is a singular lack of unanimity in the results attained. This is largely due to the fact that only in a very few cases has the process of development of the sex gland been followed in an extensive series of stages. Such work has naturally resulted in giving rise to many false and contradictory views upon these subjects.

The difficulties in the investigation of these problems are further enhanced by the fact that the sex glands are composed entirely of mesodermal tissue, in which a large part of the cells are without definite cell boundaries.

2. MATERIAL AND TECHNIQUE.

The material employed includes numerous stages in the development of the ovary and testis of the rabbit, from the 13-day embryo to and including adult stages. The pig material includes only embryonic stages, but is more complete for the period covered than is the rabbit material.

The two forms studied—rabbit and pig—are complementary in their points of special suitability for this work. Although there are minor differences in the development of the sex glands in these two forms, yet the general process is essentially the same. In general the pig is the more instructive form for a study of the early stages of embryonic development, while the rabbit furnishes material better suited for the study of the post-embryonic stages.

The following tables indicate the stages studied, the sex of the specimen and the number of series cut in each case. The stage of development is indicated in the rabbit by the number of days and in the pig by the length of the embryo.

RABBIT.

		Indifferent.	Female.	Male.
Embryonic Stages.	13 D.	1	..	..
	14½ D.	..	3	2
	16 D.	..	2	2
	17 D.	..	4	2
	18 D.	..	2	..
	19 D.	..	2	..
	21 D.	..	2	3
	23 D.	..	1	1
	25 D.	..	1	..
	26 D.	..	1	3
	At Birth.	..	2	4
After Birth.	3 D.	..	..	2
	8 D.	..	..	2
	10 D.	..	2	..
	13 D.	..	1	..
	17 D.	..	1	..
	24 D.	..	..	1
	25 D.	..	1	..
	31 D.	..	1	..
	37 D.	..	1	..
	45 D.	..	1	..
	50 D.	..	1	..
	78 D.	..	1	..
	85 D.	..	2	..
	93 D.	..	1	..
	100 D.	..	1	..
	130 D.	..	1	1
	140 D.	..	..	1

One each of the following stages of adult rabbit ovaries:

6-months-old virgin.
Old individual, 3 months since last pregnancy.
3½ days pregnant.
3¾ " "
7 " "
13 " "
14½ " " 1st pregnancy.
16 " "
17 " "
22 " "
During lactation.

PIG EMBRYOS.

Stages.	Indifferent.	Female.	Male.
0.6 cm.	1	..	..
0.7 cm.	1	..	..
0.8 cm.	1	..	..
0.9 cm.	1	..	..
1 cm.	1	..	..
1.1 cm.	1	..	..
1.25 cm.	1	..	..
1.33 cm.	1	..	..
1.4 cm.	4	..	..
1.5 cm.	2	..	..
1.6 cm.	2	..	..
1.7 cm.	4	..	..
1.8 cm.	..	3	2
2.5 cm.	..	2	1
3 cm.	..	3	1
3.5 cm.	..	1	..
4 cm.	..	2	1
5 cm.	..	2	..
5.7 cm.	..	2	1
7.5 cm.	..	1	1
8 cm.	..	1	4
8.5 cm.	..	3	3
10 cm.	..	2	2
13 cm.	..	1	2
13.5 cm.	..	1	..
15 cm.	..	1	2
18 cm.	..	1	1
20 cm.	..	1	1
25 cm.	..	1	1

NOTE.—The pig embryos were measured from the cervical to the tail bend in all stages up to 5 cm. length, when the measurement was taken from the base of the tail to the top of the head. This change was made for practical reasons of precision. The 5 cm. stage would be about equivalent to the 4 cm. stage.

The material was, in practically all cases, fixed in Flemming's fluid and stained with Heidenhain's iron hæmatoxylin with a counter stain of Säurefuchsin, Orange G. or Bordeaux red. Sections were cut to a thickness of $6\frac{2}{3}$ μ or in a few cases 10 μ and were mounted in series. It was, of course, necessary in all cases to section the anterior part of the mesonephros together with the sex gland itself.

An account of the earlier literature upon this subject would be useless repetition, since we have such valuable and extensive reviews as those given by Waldeyer, 70 and 02, Born, 94, Coert, 98, Winiwarter, 00, Bouin, 00, Mihalkovics, 85. In the general summary reference will be made to some of the more recent and important works bearing upon these problems; but no attempt will be made to attain completeness in a consideration of the earlier literature, a very large part of which is of merely historical value.

II. GENERAL TOPOGRAPHY.

The orientation of the various organs to be considered may be well understood from a study of the pig embryo of 2.5 cm. length (Text Fig. 1 and Plate I, Fig. 1). The mesonephra are a pair of elongated laterally compressed bodies attached to the dorsal body wall on each side of the mesentery. Their long axes diverge anteriorly and converge posteriorly. They are prominent structures, extending three-fourths of the length of the abdominal cavity, being closely united to the dorso-median part of its wall by their short, broad mesenteries. Each mesonephros is flattened on its median face while the lateral face is convex. A sharp ridge extending the entire length of the medio-ventral face marks the course of the Wolffian and Müllerian ducts, the latter being ventral to the former. The genital ridge is situated on the median surface of the mesonephros and extends its entire length immediately ventral to the mesentery. It is covered by a thickened layer of epithelium continuous with the general peritoneal lining of the abdominal cavity, yet differing from it in that its component cells are columnar instead of flattened, and are closely crowded together.

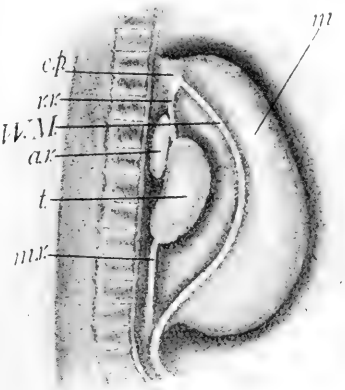


FIG. 1. Mesonephros and associated structures. Pig embryo of 2.5 cm. length. *a. r.*, adrenal body; *e. p.*, epithelial plate; *m. r.*, mesenteric ridge; *r. r.*, rete ridge; *t.*, testis; *W. M.*, Wolffian and Müllerian ducts.

Three distinct regions may be distinguished in this genital ridge, each of which occupies, roughly speaking, one-third of its length. Named in their order, they are: (1) the rete; (2) the sex gland; (3) the mesenteric ridge.

The anterior end of the rete is a low plate of thickened epithelium in which lies the opening of the Müllerian duct. Posterior to this plate, the rete assumes the form of a slender, low ridge that terminates at the anterior end of the sex gland.

In both male and female of this stage, the sex gland is cylindrical, and rounded at both ends. It projects well into the body cavity, being united to the mesonephros along its entire length by a relatively narrow mesentery.

For the posterior third of the genital ridge I suggest the term mesenteric ridge. This diminishes in height from its anterior to its posterior end, which grades off into the general peritoneal covering of the mesonephros.

A transverse section of the epithelial plate in which the Müllerian duct takes its origin, shows it to be similar to that investing the remainder of the genital ridge. At the dorsal edge of the plate are seen more or less solid invaginations, the rete cords, while the opening of the Müllerian duct is situated in the ventral part. It appears as a hollow invagination clothed with cells much like those of the epithelial plate, from which they are undoubtedly derived, as can be easily seen from a study of earlier stages.

The rete consists of a series of cords embedded in a loose stroma. Their proximal ends are directly continuous with the peritoneum while their distal extremities lie deep in the stroma, in some cases reaching to the Malpighian corpuscles with which they are frequently in direct contact.

The rete cords penetrate into the sex gland a short distance behind its anterior end. This point, termed the hilum, is morphologically the anterior end of the sex gland, although it appears to be situated more posteriorly in the testis, owing to a secondary flexure of that organ. The ovary, on the other hand, retains the primitive condition in this regard.

At this stage the ovary and testis can be readily distinguished, although they contain essentially the same structures, viz: Sex cords, albuginea and germinal epithelium. (1) The sex cords of the testis develop into the seminiferous tubules which, at this stage, appear as long contorted anastomosing and branching cords of cells. Their homologues in the ovary are termed the medullary cords. These have all

the essential characters of the seminiferous tubules save for the fact that they are by no means so well-developed nor so extensive as those structures. (2) A zone of connective tissue separates the sex cords from the peripheral peritoneal investment of the sex glands. It is compact in the testis, while in the ovary it is loose, broad and irregular in outline, forming only an incomplete barrier between the peritoneum and medullary cords. In both ovary and testis this peripheral connective tissue zone is continuous with masses of loose connective tissue (stroma) packed in between the sex cords. I shall refer to it as the albuginea in both testis and ovary, although that term is usually applied to it in the testis alone. (3) The peritoneal layer is thin in the testis and its component cells are flattened. Quite a different condition prevails in the ovary where it is decidedly thickened and is seen to be giving off cords of cells from its inner edge. These are the so-called egg-tubes of Pflüger. They are in some instances continuous with the medullary cords, although such cases are rather rare, the two sets of structures being usually distinctly separated by the albuginea.

The posterior third of the genital ridge (mesenteric ridge) need not be considered further save to note that it becomes more elevated in later stages and takes on a more decided mesenteric character.

In the mesonephros there soon appear processes of degeneration that bring about decided changes. Even in the embryo 3.5 cm. in length there is seen a commencement of degeneration in certain tubules in its anterior portion. This process continues during succeeding stages, chiefly affecting the Malpighian corpuscles, but sparing from 10 to 12 of the tubules destined to form the rete efferentia of the testis, but which later degenerate in the female. To such an extent has this degeneration process been carried on in the 10 cm. embryo that the portion of the mesonephros lying anterior to the hilum is shrunken and the investing peritoneum thrown into wrinkles. Degeneration of the portion posterior to the hilum has just begun at this stage. In the female, the shrinkage of the anterior part of the mesonephros has caused the anterior ends of the Müllerian and Wolffian ducts to be bent over the ovary in a dorsal direction to such a degree that sections through this region show these ducts to be cut through twice (Fig. 3). After this, the degeneration process rapidly reduces the mesonephros, until, in the 20 cm. embryo, it consists of little more than a mere mass of connective tissue containing a few scattered glomeruli and uriniferous tubules, the vasa efferentia in the testis alone being spared.

III. OBSERVATIONS UPON SUCCESSIVE STAGES OF DEVELOPMENT IN THE PIG.

0.7 cm. Embryo.—The mesonephros is covered by a more or less distinct peritoneal layer, which is not clearly differentiated from the stroma, except in the dorsal and lateral portions, but becomes increasingly distinct on the medio-ventral surface, where the genital ridge later takes its origin. The transition is, however, a very gradual one and the differences slight. There is a rather loose vascular mesenchyme tissue that fills in the space between the peritoneum on the one hand and the Malpighian corpuscles and mesonephric tubules on the other.

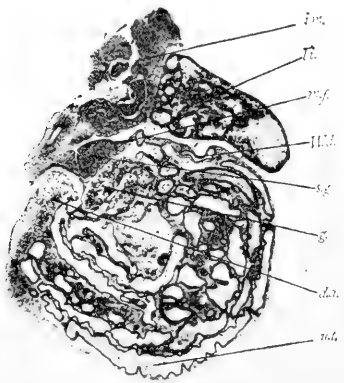


FIG. 2. Transverse section of mesonephros and associated structures. Pig embryo 1.4 cm. length. *d. a.*, dorsal aorta; *g.*, glomerulus; *i. m.*, mesentery of the intestine; *li.*, liver; *m. f.*, mesenteric fundament; *s. g.*, sex gland; *u. t.*, uriniferous tubule; *W. d.*, Wolffian duct. × 26.

The cells of both the peritoneum and underlying mesenchyme do not have definite boundaries, appearing in this, and in later stages as well, to form a continuous protoplasmic network, to which the nuclei give character by their more or less definite arrangement. A region of the peritoneum extending from the base of the mesentery one-third the distance to the Wolffian duct is of particular importance, since it is the rudiment from which the genital ridge takes its origin (Text Fig. 2 and Plate I, Fig. 3). A point about opposite the twentieth glomerulus marks the boundary between the future sex gland and the rete.

In the region of the genital ridge (Plate I, Fig. 3), as defined above, the greater part of the nuclei are of various shapes and sizes and stain rather deeply with hæmatoxylin. The nuclei of the peritoneum are closely packed together and are usually elongated by mutual pressure. They rest upon a loose felt-like basement membrane, which is formed by the interlacing of numerous slender branching protoplasmic fibrils given off by both the peritoneal and stroma cells. The peritoneal origin of the stroma is clearly indicated at many points where mutual pressure of the peritoneal cells is crowding them through the basement membrane, which has, in fact, disappeared at such spots as a result of this process. The positions and angles of inclination of the columnar nuclei give satisfactory evidence on this point. The presence of numerous mitotic figures in the peritoneum indicates a

rapid multiplication of its cells. On the other hand the stroma cells divide with far less frequency.

As might be expected from the above, the stroma cells are practically identical with the peritoneal cells from which they are originating. In general their nuclei tend to assume a more rounded shape.

Here and there in both peritoneum and stroma one finds cells quite different from those described above. These have clearly marked boundaries, lightly staining cytoplasm, a centrosphere and a centrosome. The large, round nucleus contains prominent nucleoli, usually two in number, and also a chromatin network of slender strands quite different in appearance from the rather granular irregular chromatin masses of the peritoneal and stroma nuclei. These primitive ova are so rare that one must hunt through as many as seven or eight sections in order to find one. They divide by mitosis, as a result of which division they are found to occur in small groups.

The inner boundary of the mesenchymal portion of the sex gland rudiment is formed by the capsules of the Malpighian corpuscles. The component cells of the capsules resemble those of the stroma in their lack of definite boundaries and in the character of their nuclei, being distinguished from the latter chiefly by the darker color of their cytoplasm.

The rudiment of the rete (Plate I, Fig. 2) is essentially like the sex gland rudiment save for the fact that the basement membrane of the peritoneum is somewhat less distinct in the former than in the latter and the primitive ova are not quite so numerous; these differences are probably due to the fact that the tissue of the sex gland rudiment is more dense than that of the rete rudiment.

0.8 cm. Embryo.—In this stage, the mesonephros is found to have almost doubled in size; for this reason there has been little thickening of the rete and sex gland rudiments. The number of primitive ova has greatly increased and many clear cases of mitosis are found among them. The basement membrane of the peritoneal layer has become more clearly defined in both rete and sex gland rudiments, yet it is still broken in spots where cells are being proliferated into the underlying stroma, sometimes forming chains of two, three or four cells.

1.0 cm. Embryo.—The mesonephros has become half again as broad in this stage as in the preceding one, and has also increased in the dorso-ventral dimension.

The rete rudiment has not grown in thickness, yet the peritoneal cells are seen to be rapidly dividing by mitosis. This results in a

crowding which in some places is so great as to bring about the formation of actual peritoneal invaginations which extend into the stroma and frequently come in contact with the attenuated capsules of Bowman.

Peritoneal invaginations arising in the sex gland rudiment (compare Plate II, Figs. 5 and 6) by the same process of crowding are more diffuse, and more numerous than in the rete. Another difference between these two regions of the genital ridge is found in the fact that the sex gland invaginations do not in any case reach as deep as the capsules of Bowman, the stroma being thicker in this region than in the rete rudiment.

In many cases the stroma cells are assuming the character of connective tissue. Primitive sex cells are present in the peritoneum and in the peritoneal invaginations and stroma of both rete and sex gland rudiments. Their number has increased in the sex gland rudiment, while they have shown little or no numerical increase in the rete region.

1.25 cm. Embryo.—Although the rete rudiment has increased but little in thickness, the peritoneal invaginations of the rete region, which may now be termed rete tubules, are much further developed than in the preceding stage. The sex gland rudiment, on the other hand, has increased greatly in thickness. Its peritoneal invaginations (sex cords) have also increased in length and in number. Their nature can be best understood by referring to Plate II, Fig. 5. In this and in later stages the nuclei are still attached to the basement membrane which is in fact formed, as we have seen, from protoplasmic processes connected with them. So closely are the sex cords placed that there are very few stroma cells between them. No clear cases of such are seen in this figure. Such, however, are present and form in part the rudiment of the intertubular stroma so prominent in later stages. There is no doubt that this stroma from time to time receives additions from cells which pass through the investing membrana propria of the sex cords.

The sex cords are tubular invaginations of the peritoneum and their membrana propria are accompanying infoldings of the basement membrane as seen in earlier stages (Plate I, Fig. 4 and Plate II, Fig. 5). The sex cord nuclei are connected with the membrana propria by fine fibrils which apparently hold them in position.

In its earliest stage, this is a true process of invagination, but in the later stages it is only apparent because of the fact that the sex cords grow at their points of attachment to the peritoneum (centrifu-

gal growth). Without doubt the sex cords are homodynamous with the rete tubules.

1.4 cm. Embryo.—The nuclei of the peritoneum covering the rete are more numerous than in the preceding stage, being even more closely crowded. This has resulted in a further increase in the number of rete cords. Primitive ova may or may not occur in any given rete cord (Plate II, Fig. 6), there being apparently no regularity in this matter.

Immediately ventral to the peritoneum of both rete and sex gland there is a thickened area of stroma to which addition is constantly being made by proliferation from the peritoneum. This thickening is of no especial importance in the rete, but in the sex gland it, together with a similar but less important area dorsal to the sex gland, furnishes the connective tissue that goes to form the mesentery. The peritoneal nuclei of these mesenteric rudiments (Plate II, Fig. 7) are cylindrical, with their long axes perpendicular to the long axis of the sex gland.

The rudiment of the sex gland shows very little advance over the preceding stage in point of structure. There has been a continued growth of the sex cords, resulting in such a thickening of the sex gland rudiment as to cause it to appear hemispherical in cross-section (Text Fig. 2). As in the previous stage, the peripheral layer of cells is not marked off from the underlying sex cords attached to it because of the fact that it is still adding to the latter by rapid proliferation.

The capillary blood-vessels and stroma cells already noted are quite evident in the interspaces between the sex cords. Here and there the walls of these capillaries show spindle-shaped nuclei which are far more attenuated than are the stroma cells. The latter are most numerous at the distal (inner) ends of the sex cords where they form a loose layer separated from the capsules of Bowman by a layer of attenuated, deeply-staining connective tissue cells which have their origin in the mesenteric fundaments already described. Posteriorly the sex gland gradually shades off into the mesenteric ridge, the sex cords becoming fainter and fainter and the primitive sex cells decreasing in number. The last named are found to occur almost at the posterior extremity of the mesonephros, where the genital ridge exists only as a strip of tissue along which the peritoneum and underlying stroma are thicker and denser than ordinary. There is an equally gradual transition from the sex gland to the rete. In following the sex gland into the rete region, the first sign of transition from the

former to the latter is noted in the nearer approach of the peritoneal invaginations to the capsules of Bowman. They become less crowded and the genital ridge decreases in height.

1.5 and 1.6 cm. Embryos.—The sex gland increases in volume to such an extent that in the 1.6 cm. stage it appears circular in cross-section. It is attached to the mesonephros by its mesentery which is now much narrower than the sex gland. The latter appears to have been constricted off from the surface of the mesonephros by lateral furrows. This, however, is not the case, because measurements show the mesentery to be as broad as the base of the sex gland of the 1.4 cm. stage. The constriction is apparent, not real. In reality the centrifugal growth of the sex cords has caused the sex gland to expand on all sides until it is now cylindrical in shape, instead of appearing as a slight elevation above the surface of the mesonephros as in preceding stages.

The sex cords are becoming longer and more contorted. Together with the growth in extent they become more clearly defined. The increase in the surface of the peritoneum caused by the expansion of the organ has not been accompanied by the formation of new cords, hence space is left in which many sex cords become arranged parallel to and immediately beneath it.

There appears the beginning of a most important process by which the sex cords become separated from the peritoneum through the development of the albuginea (Plate III, Fig. 8). The nuclei of the cords at their points of attachment to the peripheral cell layer (peritoneum) begin to elongate and in many cases to assume the appearance of connective tissue nuclei, while their cytoplasm is drawn out into slender strands that stretch across the necks of the sex cords. At the same time, a basement membrane is forming beneath the peritoneum dividing it from the elongated cells just described.

The mesentery is composed of cells derived from the dorsal and ventral mesentery fundaments, their rather irregular arrangement being disturbed by the ingrowth of a number of blood-vessels.

At this stage appears the peritoneal invagination that forms the Müllerian duct. It arises in the ventral part of a plate of thickened epithelium which forms the anterior end of the genital ridge. Exactly similar invaginations forming the most anterior rete tubules arise in the same epithelial plate immediately dorsal to this rudiment of the Müllerian duct. From the foregoing it seems fair to assume that the Müllerian duct is homodynamous with the rete tubules and sex cords.

1.7 cm. Embryo.—In this stage the sex cords become completely

separated from the peritoneum (Plate III, Fig. 9). As we saw above, this was foreshadowed in the 1.6 cm. stage by the transformation of the basal nuclei of the sex cords into elongated connective tissue elements. They remain attached to the membrana propria, which in almost all cases becomes ruptured and allows them to lie free between the peritoneum and the intact inner portions of the sex cords. They now form a connective tissue layer (albuginea) separating the sex cords from the peritoneum. Here and there one can see a sex cord that still remains attached to the peritoneum, and it is not at all difficult to find portions of the membrana propria to which a number of the connective tissue cells are still attached. The basement membrane shown in the preceding stage to be forming at the places where these sex cords are breaking away, has become completely formed except at a few points where the sex cords are still attached. The connective tissue nuclei formed in the manner above described are very similar to the mesenteric nuclei. This fact has led many to claim that the albuginea is composed of nuclei that immigrate from the mesenteric fundaments. We cannot hold this view in the face of the facts above noted. Further substantiation of the view of development *in situ* is furnished by the fact that nuclei exactly like those forming the albuginea are found in the peritoneum, being no doubt formed by the same process that produced the albuginea tissue.

The two sexes cannot be clearly distinguished from one another at this stage, the process above outlined taking place in both ovary and testis. The separation of medullary cords is, however, not quite so complete in the ovary as in the testis, yet this can hardly serve to sharply distinguish the sexes at the stage now under consideration.

The rete strands are quite well developed at this period, being long and somewhat contorted (see Plate IV, Fig. 11). They usually take a course more or less nearly parallel to the peritoneum to which they still remain attached at their points of origin. In general, they grow posteriorly, those found at the posterior end of the rete regions extending into the anterior part of the sex gland. Along their course they frequently touch the capsules of Bowman, some of them growing straight inward from the peritoneum in such a manner that their tips come directly in contact with the Malpighian corpuscles, thus appearing to form, in some cases, a part of their epithelial walls. This closeness of union is frequently sufficient to deceive one into considering them to take their origin from the capsules of Bowman.

At the boundary between sex gland and rete there is a transition area in which the peritoneum becomes considerably thickened. Pos-

terior to this it is found to send numerous short projections into the underlying stroma, and further back, these assume the character of closely-crowded sex cords. At this place the rete tubules are few in number and arise exclusively along a line very close to the mesentery. They can be distinguished from sex cords only by their isolation and by their greater length.

In general the rete tubules are made up largely of cells without definite boundaries and in all other regards like those of the peritoneum from which they originate. Only occasionally does one find a primitive sex cell.

1.8 cm. Embryo.—Sexual differentiation is not yet clearly established, although, in a vague way, the general distinctions mentioned in connection with the 1.7 cm. stage are to be taken as criteria.

The sex cords stand out in greater contrast to the stroma owing to the fact that the cytoplasm of the component cells is much denser than in the preceding stages (Plate IV, Fig. 12). In some places these cords show a central lumen. This is not due to any regular process of lumen formation, but has significance only in showing that there is in each sex cord a line of weakness or rudimentary lumen which owes its existence to the fact that these cords originated by a process of invagination of the peritoneum.

Exclusive of the primitive sex cells, there are two extreme types of nuclei common to the sex cords, intercordal stroma and albuginea. Those of the one kind are small and elongated, taking a deep diffuse stain (Plate IV, Fig. 12). The nuclei of the other type are larger, clearer and more rounded. There are all intermediate forms between these two extremes. The larger nuclei predominate in the peritoneum, the smaller variety characterize the albuginea, while the two kinds appear in about equal number in the sex cords and in the intercordal stroma. The marked similarity between the nuclei of the sex cords and stroma is not surprising when one considers the fact of their common origin. In the sex cords we shall term these the germinative cells in contradistinction to the primitive sex cells. Transition forms are found to unite the two distinct types of cells, thus showing that certain of the germinative cells are being transformed into primitive sex cells. The medium-sized germinative cells are probably the most primitive; these form the sex cells on the one hand and on the other the connective tissue cells. It is interesting to note that the nuclei of many germinative cells are dividing by amitosis.

The germinal epithelium of the sex gland of the 1.8 cm. embryo does not contain any primitive sex cells clearly differentiated as such.

It is significant to note that one finds here certain transition forms which link the usual type of peritoneal cells with the primitive sex cells found in the sex cords. In some cases these transition nuclei show much the same characters as regards chromatin and nucleolus as do the nuclei of the primitive sex cells, yet they differ in shape and size. It should be noted, in this connection, that the peritoneal layer is almost completely separated from the sex cords by the albuginea.

There are numbers of spherules of fat in the peritoneum covering the sex gland. These evidently indicate a process of fatty degeneration that seems to attack the cytoplasm of the cells and later to destroy a few of the nuclei, resulting in giving to the peritoneum a ragged appearance, there being large gaps where the cells have been destroyed.

2.5 cm. Embryo.—Sex differentiation is very strikingly shown in this most important stage. In the testis the albuginea has become thicker and denser than in the preceding stage. At the same time, the peritoneum has become flattened and is definitely separated from the albuginea by a distinct basal membrane. It contains no primitive sex cells. The peritoneal covering (germinal epithelium) of the ovary has become thickened and has even begun to send a few slender cords of cells into the loose underlying albuginea. These are the cords of Pflüger. They are, in many cases, loosely connected with the ovarian sex cords which we shall hereafter designate as medullary cords.

The process of fatty degeneration noted in the peritoneum of the preceding stage is still taking place in both ovary and testis, and has even extended to the sex cords in which large numbers of fat spherules appear. These occur almost exclusively in the syncytial cytoplasm of the germinative cells. They are most numerous in the portion of the sex cords furthest from the mesentery. Their fatty nature seems pretty evident from the fact that they are stained black by osmic acid and also from the spherical form that they assume.

The medullary cords are quite shrunken, being in most part clumped together in an irregular mass lying near to the mesentery. Cell degeneration occurring in them is not balanced by sufficiently rapid cell division. The primitive sex cells are surrounded by the undifferentiated cells that we have been terming germinative cells. This term, however, should henceforth be applied strictly to these cells in the seminiferous tubules alone. All resemblance to a tubular condition is lost, the medullary cords appearing in the form of masses of cells with no evidence of a very regular arrangement.

Numerous stroma cells are found to have become highly modified. The cytoplasmic portions increase in amount, becoming clearly marked off from surrounding cells, a centrosphere and centrosome appear, and the nucleus becomes rounded, while its chromatin network stains deeply. In general they assume a certain resemblance to primitive sex cells, yet the nucleus shows marked differences in its smaller size and more deeply-staining chromatin network. They differ also in the fact that their cytoplasm becomes granular and in later stages contains droplets of fat. These modified stroma elements are the interstitial cells. They are very numerous in the testis and very rare in the ovary. In both sex glands they divide by mitosis. A large portion of the stroma nuclei do not undergo this transformation into interstitial cells, but become elongated and take on the character of connective tissue. In all probability these are the cells whose nuclei were smallest in the preceding stage where a difference in size and appearance of the stroma nuclei was noted. Particular stress should be laid upon the fact that the interstitial cells appear contemporaneously with the process of fatty degeneration in the sex cords and that they show points of resemblance to the primitive sex cells. The latter point is particularly significant in view of the fact that in the 1.8 cm. embryo the nuclei of the stroma were to all appearances similar to those of the germinative cells of the sex cords which were in some cases developing into primitive sex cells. This would lead to some very attractive hypotheses; but one should be cautious about drawing hasty conclusions from such points of mere resemblance.

3 cm. Embryo.—There are no essential differences between the rete of ovary and testis. The rete cords are still being formed, their points of connection with the peritoneum persisting along the entire length of the rete rudiment in both sexes. Another point common to both sexes is the degeneration of certain Malpighian corpuscles of the anterior part of the mesonephros. Those lying nearest to the rete cords are especially affected, suffering a decrease in size and a consolidation of the capillaries contained in them.

Primitive sex cells are being formed in the rete cords from the syncytial cells that have retained the primitive character exhibited by the peritoneal cells, from which these cords arise. This development of undifferentiated peritoneal derivatives to form primitive sex cells is probably homologous with the process by which the germinative cells of the seminiferous tubules of the testis and the cells of the cords of Pflüger of the ovary are being transformed into primitive sex cells. The sudden impulse to renewed activity in the

formation of these cells apparently affects both rete and sex gland at the same time. It is barely possible that the presence of fatty spherules so evident in the 2.5 cm. stage may be in some manner correlated with the active formation of primitive sex cells in the seminiferous tubules, cords of Pflüger and rete cords, all of which structures have been shown to be homodynamous. Such a hypothesis would, however, require more evidence for its proof than we have yet found.

The fat globules so numerous in the sex gland of the 2.5 cm. embryo have almost entirely disappeared from all save the interstitial cells of the testis, in the fat globules of these there has been an increase which may or may not be correlated with their disappearance in the seminiferous tubules. Whatever loss of cells may have taken place in the seminiferous tubules at the preceding stage has been compensated for by a process of rapid cell division. This, together with the transformation of germinative cells, has resulted in a decided increase in the number of primitive sex cells.

The peritoneum of the testis has become still further flattened, and its fatty spherules have almost wholly disappeared.

The albuginea nuclei have become more attenuated than in previous stages, yet they do not differ essentially from certain other connective tissue elements of the stroma, many of the more attenuated of which are seen to become applied to the membrana propria of the seminiferous tubules in such a manner as to form thin connective tissue sheaths.

The interstitial cells are an extremely important constituent of the testis, occupying the interspaces between the seminiferous tubules (see Plate IV, Fig. 13). In the ovary, on the other hand, they are very sparse. In the place of them one finds great masses of loose connective tissue, filling the interspaces between the other ovarian tissues.

3.5 cm. Embryo.—This stage will be noted chiefly to record the reduction in the cytoplasm of the interstitial cells of the ovary. Not only has the cytoplasm of these sparse cells become shrunken, but the centrosome and centrosphere have almost disappeared. Both primitive sex cells and follicle cells of the medullary cords are suffering extensive degeneration. This continued process of degeneration is even more marked in the cortex and cords of Pflüger, which are now just beginning to assume importance.

4 cm. Embryo.—There is an interesting process of karyolytic degeneration that appears in the rete cords of this stage. The chromatin of the nuclei so affected gathers together in a rounded solid

mass which is finally set free in the cytoplasm by the rupture of the nuclear wall. It now breaks up into irregular fragments which finally become more or less rounded and eventually disappear. Here and there one finds nuclei of the seminiferous tubules and cords of Pflüger which degenerate in the same manner. This process takes place very extensively in later embryonic stages.

In both sexes the rete cords along at least three-fourths of the length of the rete region have become separated from the peritoneum by a layer of stroma. It was impossible to determine whether this process is analogous to the separation of the sex cords from the peritoneum covering the sex gland.

Attention has already been called to the degeneration of certain Malpighian corpuscles of the anterior end of the mesonephros in the 3 cm. stage. In the particular specimen now under consideration (4 cm. embryo) this process has continued, affecting eight of the most anterior corpuscles. The remaining ten or twelve corpuscles between the degenerate ones and the hilum of the testis are, as a whole, quite normal. Certain of the more peripheral of these intact Malpighian corpuscles send out short evaginations that come in contact with corresponding processes from the mass of rete cords and fuse with them (Plate V, Fig. 15). In this manner preparation is made for the establishment of a subsequent connection between the mesonephric tubules and the rete cords. Connection is also no doubt established without the aid of these evaginations in cases where the rete tubules press tightly against the capsules of Bowman. The number of the above described evaginations arising from each Malpighian corpuscle varies decidedly. In many cases there are none at all; in others there are as many as three.

Smaller evaginations from the capsules of Bowman were found in a 3 cm. embryo.

5.7 cm. Embryo. Ovary.—One is struck by the very close resemblance between the medullary cords and the cords of Pflüger. They are practically identical, position alone serving to distinguish them. The medullary cords (Plate III, Fig. 10) lie in the central axis of the sex gland, separated by a zone of connective tissue from the cords of Pflüger which project inwards from the peritoneum. Both elements are in large part composed of primitive sex cells—in fact there are but few small, deeply-staining nuclei which may be identified as those of rudimentary granulosa cells. The latter have no well-defined limits, being in every regard similar to the cells of the peritoneal layer from which the cords of Pflüger arise.

Fatty degeneration has almost ceased in the medullary cords and cords of Pflüger.

7.5 cm. Embryo. Ovary.—A few of the sex cells of the cords of Pflüger have undoubtedly developed into the condition of oöcytes because of the fact that their chromatin threads have taken on the synapsis form described by Winiwarter, oo, in the rabbit. Corresponding synaptic stages are also found in the medullary cords, thus bringing out the close homology of the two structures.

The cords of Pflüger have become elongated and have at the same time branched and anastomosed to form a network in a manner quite like that of the seminiferous tubules in the testis. The resemblance is still further heightened by the fact that the cords of Pflüger are invested with a connective tissue layer formed by attenuated connective tissue cells of the stroma. The same is true of the medullary cords.

We might homologize these three structures by considering the seminiferous tubules and medullary cords as exactly homologous structures, while the cords of Pflüger constitute a second series of invaginations in all respects homologous with the medullary cords save as regards the time of origin.

Testis.—The structure of the testis is essentially the same as in earlier stages. There has been a progressive increase in the extent of the system of seminiferous tubules, which has been brought about by the continued growth and branching of those already laid down previous to their separation from the peritoneum (1.7 cm. embryo). The nuclei of the germinative cells are attached to the basement membrane by strands denser than the surrounding cytoplasm. This relation to the basement membrane is exactly similar to that of the peritoneal cells in the earliest stages (0.7 cm. embryo). The primitive sex cells are increasing in number by two processes, namely: (1) division by mitosis of those already present in earlier stages; (2) transformation of germinative cells into sex cells. All stages in this transformation process can be noted, any transverse section of the testis at this stage (Plate IV, Fig. 14) showing a complete series of transition forms. The same may be seen in embryos earlier and later than this, namely, from 3 cm. to 13 cm. in length. Primitive sex cells occur in the rete cords of both male and female, those of the male being apparently in the same stage of development as are those of the seminiferous tubules. This is not true in the female at this stage, owing to the fact that the primitive sex cells of the cords of Pflüger and medullary cords have developed precociously, outstripping those of the rete ovarii.

8.5 to 10 cm. Embryo.—Up to the stage when the embryo is 8.5 cm. in length, the rete cords extend but a short distance straight in from the hilum in the case of both ovary and testis, and are similar in both sexes, the primitive sex cells of the rete ovarii becoming larger than those of the rete testis.

The embryo of 10 cm. length shows the rete testis to have rapidly developed an axial core of loose connective tissue that fills in the space central to the free tips of the radially directed seminiferous tubules. In the female, on the other hand, the rete ovarii extends no further into the ovary than in the preceding stages, remaining in contact with the anterior end of the irregular mass of medullary cords. The rete ovarii and rete testis now follow different courses of development.

13 cm. Embryo. Male.—The cords of the rete testis have in most cases undergone a process of lumen formation. This is brought about by the drawing apart of the cells from the axis of the cords. As already shown, these rete cells are attached to the ensheathing membrana propria, hence the lumen is formed by a very simple process by which they are made to separate along the line of greatest weakness (axis of cord). These rete tubules branch and anastomose quite like the seminiferous tubules. Their homology to the latter is still more clearly shown by the great similarity in the component cells of the two structures.

The rete tubules contain a few primitive sex cells (Plate VI, Fig. 21) exactly like those found in the seminiferous tubules. These are nothing new, as we have seen them to occur in the rete tubules of the very earliest stages. Another point of similarity is found in the character of the epithelial cells of the rete tubules which are in every regard similar to the germinative cells of the seminiferous tubules. Transition forms between epithelial cells and primitive sex cells do not exist in the former, while they are quite plentiful in the latter.

The rete tubules send out side branches (tubuli recti) that fuse with the inner ends of the seminiferous tubules. In this manner one rete tubule may come into direct connection with a large number of seminiferous tubules. In one section, a rete tubule was seen to send out four tubuli recti connecting with as many seminiferous tubules. The point of junction of tubulus rectus and seminiferous tubule (Plate V, Fig. 18) is easily recognized by the difference in diameter of the two elements, by the difference in arrangement of their component cells and by the presence of a lumen in the rete tubules as contrasted with the absence of such in the seminiferous tubules. In cases where connection has not been completely established between these two struc-

tures, the point of junction is marked by the persistence of the basement membrane of tubulus rectus and seminiferous tubule. These membranes are soon absorbed and the two structures are in direct continuity with one another.

Female.—The primitive sex cells are found in all parts of the rete ovarii, yet their distribution is in no sense uniform. The intra-ovarian portion contains great numbers of primitive sex cells, which show a close resemblance to those found in certain regions of the cords of Pflüger. Associated with these sex cells of the rete are other and smaller cells which are practically identical with certain cells of the cords of Pflüger destined to form the granulosa of the Graafian follicles.

As stated above, the primitive sex cells are not by any means confined to the intra-ovarian portion of the rete tissue, yet their number in the portion of the rete lying within the mesonephros is found to become less and less as the distance from the ovary increases. The same principle holds true in the male.

The medullary cords are greatly reduced (Text Fig. 3), consisting of clumps of cells containing sex cells in various stages of development, the most advanced being large oöcytes with a well-formed layer of granulosa cells. Such young follicles are rare and isolated.

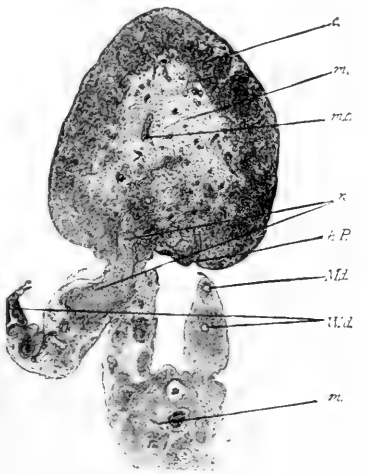


Fig. 3. Transverse section of ovary and mesonephric structures of pig embryo. Length 13 cm. *c.*, cortex; *h. p.*, hollow cord of Pflüger; *m.*, medulla; *m. c.*, medullary cord; *M. d.*, Müllerian duct; *mes.*, mesonephros; *r.*, rete ovarii; *W. d.*, Wolffian duct. ×26.

The innermost ends of the cords of Pflüger are being broken up to form follicles. These follicles are young, each consisting of a large oöcyte and a single layer of granulosa cells. The oöcytes almost invariably contain numbers of fat globules situated in their cytoplasm and especially numerous about the centrosphere, where they appear to congregate, eventually combining to form a single large mass. This appears to be without doubt a process of degeneration, leaving clumps of granulosa cells which persist for some time after the oöcytes have disappeared. Not only are these oldest sex cells being destroyed by fatty degeneration, but there is an independent process of karyolysis which destroys great numbers of younger sex cells. In addi-

tion to these two processes is that by which the fine chromatin threads in the nuclei of the oöcytes at the synapsis stage of their development frequently break down into a powder-like mass of very fine granules. This is no doubt another process of degeneration.

On the side of the ovary facing the mesonephros, there are a number of invaginations of the peritoneum (Text Fig. 3). These often appear as hollow tubules that extend for some distance into the ovary. At points along their extent they are found to be solid, their cells being similar to those of the cords of Pflüger. In fact they are to be interpreted as such. Transition regions are found in which the peritoneal lining of these hollow tubules is found to contain a greater and greater percentage of primitive sex cells (Plate VII, Fig. 25) up to the condition of the solid portions of the tubules where the lumen is entirely obliterated by the enlargement of the peritoneal cells to form primitive sex cells.

15 cm. Embryo. Female.—In this stage the above described hollow egg-tubes of Pflüger, while still most common around the hilum, are also found in the region of the cortex furthest from the mesentery. They penetrate more deeply into the tissue of the ovary than in the preceding stage, some of them extending into its very center, where they could be readily mistaken for rete tubules by persons who might have studied these structures in other forms, such as the cat. There is no mistaking their identity, however, because they bear no resemblance to the true rete tubules and because they were readily followed through the series to their point of union with the peritoneum. These invaginations may arise either from deep grooves or from the smoother surface of the peritoneum. At this stage the medullary cords are still further reduced, no young follicles being found among them.

The mass of rete tissue is now found to be constricted at its point of entrance into the ovary. Further development of the sex cells and of the intra-ovarian rete has caused the boundaries of the rete cords to become obscured.

Male.—In the male, the glomeruli connected with the rete tissue have degenerated to such an extent that the rete tubules are now found to be in almost direct contact with the mesonephric tubules. A minute description of the seminiferous tubules of this stage will serve to unify the points touched upon in the preceding pages (see Plate V, Fig. 18). They are still solid, yet their tubular nature is shown by the arrangement of the dense peripheral layer of nuclei belonging to the germinative cells. Each nucleus is attached to the

membrana propria by a cylindrical condensation of cytoplasm, frequently so short that the nucleus appears to rest directly upon the membrana propria. The axial portion of the tubule is occupied by a loose network of protoplasm. At no time do these germinative cells have definite boundaries.

There are at this stage no transition forms between the germinative cells and primitive sex cells.

18 cm. Embryo. FEMALE.—This stage shows some interesting points in the development of the intra-ovarian portion of the rete tissue. Lying in the mesentery at the hilum, it extends but a short distance into the ovary, not reaching the inner ends of the adjoining cords of Pflüger. In this intra-ovarian portion of the rete, the oögonia have in some cases developed so far as to be surrounded by well-defined follicles (Plate VI, Fig. 20). These young follicles have but a single layer of granulosa cells and are exactly like the follicles formed in the inner portions of the cortex, at this stage. With these rete follicles are found sex cells in all stages of development, likewise resembling corresponding sex cells in the cords of Pflüger. The resemblance is made more complete by the fact that the sex cells of the rete are undergoing the same process of degeneration as are corresponding sex cells in the cords of Pflüger.

The portion of the rete lying in the mesonephros has become distinctly separated from the intra-ovarian portion just described. Most noteworthy, however, is the fact that the few primitive sex cells found in it have not developed beyond the original condition which they exhibited in the early stages of development.

Few of the open tubes of Pflüger exist as such at this stage, most of them having become transformed into solid cords of cells such as characterize the cortex as a whole.

20 cm. Embryo. FEMALE.—A careful study of the inner ends of the cords of Pflüger shows that many of the oöcytes of the young primitive follicles have disappeared as a result of the process of degeneration already described. The granulosa cells are apparently not affected, but persist in solid elongated clumps, similar to the remains of the medullary cords found scattered through the axial portion of the ovary.

All the primitive sex cells and oöcytes of the rete tissue have disappeared, leaving only the granulosa cells and their homologues.

25 cm. Embryo. FEMALE.—At this stage the surface of the ovary is found to have become wrinkled and irregular. The cords of Pflüger form a thick, dense, cortical layer, within which is the medullary por-

tion of the ovary, made up of loose connective tissue (stroma) which extends between the cords of Pflüger in the form of strands and plates having a texture denser than that of the central mass. These strands are continuous with a sub-peritoneal layer of connective tissue that separates the cords of Pflüger from the peritoneum, thus putting an end to their further growth at the expense of the latter. Here and there a slender ingrowth from the peritoneum is still found to pierce the connective tissue layer, yet these are of slight importance. I am not prepared to say whether they assume greater importance in later stages.

The cortex contains sex cells in all stages of development, from the very young oögonia of the peripheral region to the small follicles in its innermost edge. The remains of the medullary cords and of the intra-ovarian portion of the rete are still present in the medullary region and are found to be in practically the same condition as in the 20 cm. embryo. Not a sign of sex cells is to be seen in either the intra- or extra-ovarian portions of the rete.

The rete tissue is much more extensive in this stage than in the 20 cm. stage. There it was again more extensive than in the preceding (18 cm.) stage). Although these observations would seem to point to its growth after the degeneration of the sex cells, one should not lay too much stress upon this point. These seemingly conclusive facts may be conditioned by the great variability universally seen to exist in vestigial structures. A study of the rete cells failed to reveal extensive nuclear division in the above stages.

IV. DETAILS OF DEVELOPMENT IN THE RABBIT.

13-Day Embryo.—There is at this stage no observable difference between the structure of the sex gland and rete rudiments. This stage corresponds with the 0.8 cm. stage of the pig. One is struck with the vagueness of the basement membrane of the peritoneum both in this and in succeeding stages of the rabbit, yet it is as truly present as in the pig embryos where it appears with remarkable distinctness. Many of the cells of both stroma and peritoneum are found to be quite irregular in shape, in many cases even amœboid. Frequently they appear to be dividing by amitosis. It is very difficult to decide whether this be merely apparent or real. This point deserves special study, as it is of prime importance. A few figures of mitosis appear here and there. Primitive sex cells are present, though rare, occurring either in the peritoneal layer or beneath it. They are to be distinguished from the surrounding peritoneal and stroma cells by the same

criteria noted in the pig embryo. Both rete and sex regions are found to contain them.

14½-Day Embryo.—At this stage the sex gland rudiment is easily distinguishable from the rete portion of the genital ridge. It is hemispherical in transverse section, having attained a marked increase in height over the preceding stage by multiplication of the cells of the peritoneum and of the stroma cells which are manifestly derived from it. Sex cords are well formed, as in the 1.4 cm. stage of the pig, being likewise continuous with the peritoneum from which they were formed by a process of invagination. Although the cords appear with a fair degree of clearness, the rabbit is by no means so favorable a subject for the determination of the manner of their formation, as is the pig.

The rete portion of the genital ridge is quite low in comparison with the rudiment of the sex gland. Here one finds certain scattered diffuse cords projecting from the peritoneum into the underlying stroma, each invested by a membrana propria continuous with the basement membrane of the peritoneum. There are a few primitive sex cells in these rete tubules, but the predominating type of cells comprises those with small, oval, deeply-staining nuclei without cell boundaries, such as compose the peritoneum. These cells are attached to the membrana propria or basement membrane, as the case may be, by slender strands of cytoplasm, showing the same relation in this regard as do the corresponding cells in the pig embryo.

The nuclei of the stroma in both rete and sex gland rudiments are found to be irregular in shape, giving the appearance of undergoing division by amitosis.

16-Day Embryo.—The rete tubules of the region anterior to the sex gland can be readily detected. They lie in a mass triangular in transverse section. This is limited by the mesentery of the mesonephros, the capsules of Bowman and the peritoneum. In places the rete tubules can be seen growing in from the peritoneum and branching in the stroma. Each has a rudiment of a lumen which opens into the body cavity on the one hand, and on the other extends for a short distance into the interior of the tubule. These rete tubules can be found along the entire length of the rete rudiment and back beneath the sex cords of the rudimentary sex gland. In this region—the anterior end of the sex gland—it is difficult to distinguish the rete tissues from the underlying layer of connective tissue cells that separates them from the Malpighian corpuscles. The rete nuclei differ from those of overlying sex cords in that they are slightly smaller, more irregular and more deeply-stained than the latter. These rete nuclei are not to be dis-

tinguished from the stroma nuclei nor from those of the peritoneum, all of the above named being amœboid in shape, and giving the appearance of dividing by amitosis.

A few large, well-marked, primitive sex cells are found in the rete tubules, beneath the sex gland and in those lying well within the anterior portion of the mesonephros in front of the sex gland.

Testis.—In the anterior end of the testis the sex cords are still attached to the peritoneum. They reach a considerable length, and are often seen to branch once or twice in their course. More posteriorly one finds cords in process of separation from the peritoneum. As in the 1.7 cm. pig embryo, the nuclei of the proximal ends of these separating sex cords are becoming elongated and are assuming the character of connective tissue elements. Finally they break away from the basement membrane to form the albuginea dividing the peritoneum from the sex cords. More posteriorly still, this process is found to have been completed.

Ovary.—In the ovary, the sex cords have not begun to separate from the peritoneum, although the introductory stages of such a process are seen. The sex cords are not so definite in outline as are those of the testis, owing to the fact that the stroma tissue separating them is not so dense as in that organ. The cells of the ovary are in all regards quite like the corresponding ones in the testis, the same small, amœboid nuclei being found in the sex cords, rete tubules, stroma and albuginea, in addition to the primitive sex cells of rete tubules and sex cords.

The peritoneum of the ovary is much thicker than that of the testis, being three cells thick in many places. Primitive sex cells are found occasionally in the innermost portions, together with intermediate forms connecting them with the ordinary peritoneal cells. The inner edge of the peritoneum is more or less irregular in outline, showing a number of short, rounded protuberances—the rudiments of the cords of Pflüger.

17-Day Embryo. Testis.—The seminiferous tubules of the testis contain primitive sex cells and germinative cells, with many transitional forms between the two. Both kinds divide by mitosis. The stroma nuclei are now more regular in shape, and no longer give the appearance of dividing by amitosis. As in the pig embryo, an investing membrane of connective tissue is found around the seminiferous tubules. This tissue has undergone a decided increase.

Cases of karyolytic degeneration are seen here and there among the cells of the seminiferous tubules and rete tubules, although it is by no means common.

The rete tubules are separated from the peritoneum save only at the anterior end of the mesonephros. They lie in a direction parallel to the long axis of the sex gland, being in some places closely applied to the capsules of Bowman. Many Malpighian corpuscles have given out evaginations that have fused with the rete tubules in the manner described in the pig embryo. The latter are distinctly separated from one another by their clear-cut membrana propria. No primitive sex cells are, at this stage, found in the rete tubules anterior to the sex gland, but they occur here and there in those underlying the anterior part of the sex gland.

Ovary.—The description of the rete testis applies to the rete ovarii, there being no essential differences between the two.

All except a very few of the medullary cords have broken away from the peritoneum. These resemble the seminiferous tubules to a certain extent, yet they have a tendency to form spherical clumps of cells which remind one of follicles.

21-Day Embryo. Testis.—The rete tissue extends beneath the testis for over half its length. It occupies the space between the somewhat excavated inner face of that organ and the mesentery. A transverse section of the testis would show the mass of seminiferous tubules to appear as a crescent between the horns of which lie the rete tubules. These anastomose, forming a mass of tissue in which the boundaries of the component cords are largely obscured. From this unified mass slender branches (tubuli recti) pass to the seminiferous tubules, with which they unite. The nuclei of the rete cells are strikingly like those of the germinative cells, this resemblance being heightened by the fact that neither kind possess cell boundaries. Here and there in the seminiferous tubules, nuclei are found to degenerate by karyolysis. Clear transition forms are found to connect the primitive sex cells with the germinative cells (Fig. 23). This stage shows the interstitial cells to be well developed. They are characterized by having well-defined limits, granular cytoplasm, centrosphere and centrosome, and a spherical nucleus somewhat smaller than that of the primitive sex cells.

Ovary.—The chief advance over the preceding stage is found in the extension of the cords of Pflüger into the loose connective tissue of the albuginea. It will be remembered that these cords were mere rudiments in the 17-day stage; now they are quite well-developed.

The medullary cords are but indistinctly separated from one another by rather sparse stroma cells which resemble the follicular cells of the medullary cords.

23-Day Embryo. Testis.—In the testis of this stage there is no important advance over the preceding stage.

Ovary.—The rete tubules have assumed the appearance of the medullary cords save for the fact that they contain no primitive sex cells—a fact which might have been noted in the 21-day embryo. There has been little essential change in their general character. The nuclei of the rete tubules and their homologues in the medullary cords are still very irregular in form, giving the appearance of being in process of division by amitosis. Undoubted amitosis occurs among similar nuclei in the cords of Pflüger (Plate VI, Fig. 22). These are destined to become the follicular (granulosa) cells of the Graafian follicles. It is possible that some of them may develop into oögonia—this point should be studied further. The cords of Pflüger are connected with the peritoneum by slender necks.

26-Day Embryo. Testis.—The rete tissue has extended the entire length of the testis. Scattered primitive sex cells are found here and there in the part lying within the testis, but are not present in the mesonephric portion.

The interstitial cells are found to occasionally divide by mitosis.

There is an extensive karyolitic degeneration of sex cells of the seminiferous tubules. Not only is the nucleus affected in the manner already described, but the cytoplasm undergoes modification as well, in that it assumes the property of staining more deeply in these degenerating cells than it does in the normal ones.

Ovary.—The medullary cords are now clearly separated from one another by rather wide intervals filled with stroma tissue. The cords of Pflüger have increased in extent and have, to a large extent, fused with one another until their original limits are marked only by dense plates and strands of connective tissue. As in the pig, we shall hereafter refer to this zone of densely-packed cords of Pflüger as the cortex, in contradistinction to the inner core of looser tissue made up of stroma and medullary cords.

Rabbit at Birth. Testis.—The rete tubules become more distinctly limited from one another and have begun the process of lumen formation simultaneously in all parts of the rete tissue. In this process the cells pull apart from the central axis of the cord which is a line of weakness due to the manner in which these cords are formed. The lumen of any given rete tubule is not continuous at first, being formed disconnectedly along the course of the cord. Each tubule is provided with a connective tissue sheath. The typical epithelial cells of these tubules form a syncytium in which the deeply-staining, columnar

nuclei are arranged side by side, usually in a single layer, although one, at times, finds a superposed layer. A few primitive sex cells occur in the portion of the rete tissue lying within the testis. A complete series of transitional forms are found to connect the primitive sex cells with the germinative cells in this as in the 17, 21, 23 and 26-day stages.

In both male and female the mesonephros proper has almost wholly disappeared, leaving a connective tissue network in whose meshes lie masses of fat. The caput epididymis (Text Fig. 4), made up of contorted tubules (rete efferentia) lined with epithelial cells, still remains. These are the persistent uriniferous tubules of the anterior part of the mesonephos, the great bulk of the tubules posterior to these having degenerated, together with a few of those which were formed in the most anterior end of the mesonephros. A few shrunken glomeruli still persist in connection with the rete efferentia.

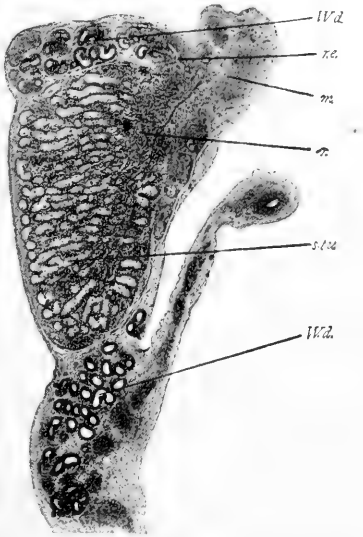


FIG. 4. Sagittal section of the testis of the rabbit, 3 days after birth. *al.*, albuginea; *m.*, mesonephric remains; *r.*, rete; *r. e.*, rete efferentia; *s. tu.*, seminiferous tubule; *W. d.*, Wolffian duct. ×28.

Rabbit 3 and 8 Days after Birth. TESTIS.—These stages are interesting chiefly because they show a marked diminution in the number of interstitial cells which, however, are still found to be in process of division by mitosis.

Rabbit 10 Days after Birth. OVARY.—The rete tubules are in all cases devoid of a lumen. They are frequently joined to hollow tubules of larger diameter which extend into the general rete mass. These are the rete efferentia. Their identity is shown by the large size of the lumen, and by the fact that the nuclei are larger than those of the rete tubules. These rete efferentia have been brought to lie within the hilum by the extension of the ovary to partially enclose the shrunken mesonephros. They probably grow into the ovary of their own account as well. The differences between medullary cords and rete tubules disappeared as far back as the 23-day stage of embryonic life.

The cortical layer is distinctly bounded from the medullary substance by a dense layer of connective tissue which follows a zig-zag

course to accommodate itself to the large rounded projections comprising the cords of Pflüger. These cords are still attached to the peritoneum by their narrow basal portions.

The medullary and rete tubules are usually two cells wide and without any trace of a lumen. Their nuclei are oval and rather uniform in size. Here and there one finds primitive sex cells singly or in groups of two to four. They are frequently in the same stage of synapsis as are the more advanced nuclei of the cortex.

In the 26-day female embryo the Wolffian duct has almost disappeared by degeneration, together with the great bulk of the uriniferous tubules. In the female, 10 days after birth, the mesonephric structures are found to have completely degenerated save for a few vestiges of uriniferous tubules lying within the rete tissue and the mesentery, posterior to the hilum. Aside from these vestiges, the mesonephros consists of loose connective tissue enclosing great masses of fat—the remains of former Malpighian corpuscles and mesonephric tubules.

13 Days after Birth. Ovary.—Follicles are forming in the cortex at this stage. They are, of course, very simple, each consisting of a large oöcyte surrounded by a single layer of granulosa cells. Exactly similar follicles are also found in the medullary cords.

Very many nuclei in all parts of the cortex and medullary cords are suffering karyolytic degeneration.

17 Days after Birth. Ovary.—The process of follicle formation has continued, resulting in the breaking up of the inner ends of the cords of Pflüger. No sex cells are found in the medullary cords from this stage on. The ova of certain of the innermost follicles have disappeared, leaving clumps of follicle cells such as are found in corresponding stages in the pig. Frequently one finds two or even more oöcytes in the same follicle.

24 Days after Birth. Testis.—This stage shows the testis to have pretty largely assumed the characters prominent in adult life. The caput epididymis is made up of the much-contorted tubules of the rete efferentia, the latter being traceable down to their points of connection with the rete testis. This, as already stated in the description of previous stages, is made up of a mass of anastomosing rete tubules from which proceed the tubuli recti that connect with the seminiferous tubules. In the posterior two-thirds of the testis, the rete is wholly surrounded by the seminiferous tubules that have closed in around it. Here and there primitive sex cells (spermatogonia) can still be found in the rete testis.

Up to the stage of 8 days after birth, there were found numerous transition forms connecting the germinative cells with the primitive sex cells. This is by no means true of the 24-day stage (Plate VI, Fig. 24), where there is a very sharp distinction between the two types of cells which are singularly uniform among themselves. This is true not only in the characters already enumerated, but also in the staining reaction as well. The germinative nuclei take the iron hæmatoxylin stain with avidity while the primitive sex cells (spermatogonia) are not affected by it at all.

25 Days after Birth. Ovary.—The cords of Pflüger are now almost entirely broken up to form large numbers of small follicles surrounded by connective tissue that has permeated the entire mass of each cord of Pflüger.

31 Days after Birth. Ovary.—It was noted in an earlier stage (17-day ovary) that certain ova of the innermost follicles degenerated leaving clumps of follicular cells. These clumps are quite evident in the 31-day stage, lying along the border between the medulla and cortex.

45 Days after Birth. Ovary.—Many of the follicles have increased in size until they have acquired as many as three layers of granulosa cells, among which appears an incipient follicular cavity. Already the stroma forms a capsular investment (theca) about each follicle, and this investment has begun to show a differentiation into a theca interna and a theca externa. The nuclei of the cells of the theca interna are rounded and the cell body has become fuller in contrast to the attenuated fibrous character of the cells of the theca externa, whose nuclei have remained elongated and in every regard like those of the general stroma tissue of which they are an integral part. Transition forms between both varieties of theca cells can be readily found. It might be well to call attention to the fact that there is a thin layer of attenuated stroma cells between the theca interna and the membrana propria of the granulosa layer. It will be termed the follicular capsule.

At this period many cells of the theca interna have developed into interstitial cells similar to those described in the testis of the pig embryo. Each has a rounded nucleus, clear cell outlines, centrosphere, centrosome and numerous fatty granules deposited in its cytoplasm. The formation of these interstitial cells is genetically connected with a process of follicle degeneration which continues from the time of the earliest formation of follicles on through adult life.

50 Days after Birth. Ovary.—The medulla becomes still more reduced in this stage by the invasion and growth of follicles in its bor-

der. The ground substance is a rather compact connective tissue in which are imbedded the slender transversely-placed medullary and rete tubules, which are quite inconspicuous and devoid of a lumen. Large open lymph spaces are formed in the stroma. Typical interstitial cells are not at all uncommon.

78 Days after Birth. Ovary.—Certain of the innermost follicles have increased greatly in size, having in some cases almost reached maturity. They encroach upon the limits of the medullary region to such an extent as to make the latter band-like in cross-section. In Plate VII, Fig. 26, is represented a portion of the ovary showing the more important tissues composing it. The granulosa cells have well-defined boundaries, being polygonal in shape as the result of mutual pressure. The nuclei are rounded and are found to divide by mitosis. The follicle is bounded externally by a clearly-defined membrana propria, external to which one finds a very thin layer of attenuated connective tissue cells. This investment (follicular capsule) is similar to that which surrounds the seminiferous tubules.

Outside of this occurs the theca interna, which is from one to four cells thick, the component cells being elongated in a direction parallel to the surface of the follicle. They are rich in cytoplasm. The large, rounded or oblong nuclei stain more lightly than do the nuclei of the granulosa cells.

The slender branching and anastomosing medullary and rete cords are distinguishable from the surrounding stroma by the clear cytoplasm and small oblong, deeply-staining nuclei of their cells which have apparently remained unchanged in character from the earliest stages onward.

Certain young follicles of a stage just before the formation of the follicular cavity have begun to degenerate, the process first affecting the oöcyte which in some cases has disappeared wholly or in part. The mass of follicular cells becomes irregular in outline, but shows no signs of degeneration. It is quite likely that certain cords of cells with nuclei larger than the true nuclei of the medullary cords in the midst of which they lie, have originated from these degenerating follicles, the resemblance between their nuclei and those of the normal follicles being very striking.

85 Days after Birth. Ovary.—The ovary as a whole has changed but little in form and size, this stage being most remarkable on account of the great increase in the number of interstitial cells. This increase is due to a very extensive process of follicle degeneration which seems to be at its height, affecting follicles in all stages of de-

velopment. The granulosa cells are the first to show signs of degeneration, the nucleus drawing up into a small globular homogeneous mass in the center of the cell. The cytoplasm changes in such a manner as to become more deeply stained than formerly and the whole cell becomes rounded. This is no doubt the process of chromatolysis described by Flemming, **85.** It certainly results in the eventual liquefaction of the cells affected.

Large numbers of connective tissue elements from the capsule and theca interna penetrate into the follicular cavity. As the granulosa cells degenerate further, the follicular cavity becomes smaller and the capsule, theca interna and theca externa contract, thus encroaching upon the follicular cavity until the latter has become greatly reduced. The above mentioned elements that have migrated into the follicular cavity from the capsule and theca interna persist after the granulosa cells have all disappeared. The cells from the theca interna later undergo fatty degeneration and finally disappear, leaving the slender connective tissue cells that had migrated from the capsule; these persist and probably remain to form part of the general stroma tissue, lying between the columns of interstitial cells, whose method of formation will be described later.

A series of fine connective tissue fibres join the follicular capsule with the theca externa passing rather obliquely between the cells of the theca interna. When the capsule closes in upon the follicular cavity these threads are drawn taut and arrange the cells of the theca interna in radial rows. The whole mass may become laterally compressed by the growth of neighboring follicles. In very advanced stages of atresia, when the follicular capsule has become reduced to a crumpled remnant lying in the midst of the cells of the theca interna, the latter lose their cell walls and become irregular in shape. In this condition they undergo a process of rapid amitotic division (Plate VII, Fig. 27), the resulting nuclei being much smaller than before this process took place. These will develop into the prominent interstitial cells as seen in later stages.

6 months' Virgin.—In the ovary of this animal (Text Fig. 5) it is possible to trace out the further development of the interstitial cells. They cease to divide and undergo a process of growth in size both of the nucleus and of the cell body. At this stage—just after division has ceased—there occurs a deposition of a substance occurring in the form of small spherules, which stain deeply with hæmatoxylin. They were found only in this one specimen and are in no wise to be confounded with the very numerous fat granules which now begin to fill the cytoplasm of these interstitial cells.

These fat granules are very characteristic of the interstitial cells. In this stage certain of these cells which have become isolated from the general mass are found to have enlarged greatly and to have become stuffed full of large fat spherules which are very readily dissolved by xylol. Each of these cells contains a large excentric nucleus with a centrosphere and centrosome (Plate VII, Fig. 28). Most of the interstitial cells are crowded together by mutual pressure and are hence prevented from attaining the full size of the one figured, which lay free in the connective tissue.

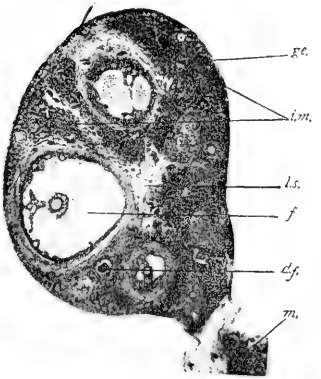


FIG. 5. Transverse section of ovary of a six-months old virgin rabbit. *f.*, follicle; *d. f.*, degenerate follicle; *g. e.*, germinative epithelium; *i. m.*, masses of interstitial cells; *l. s.*, lymph spaces; *m.*, mesonephros remains. × 24.

8 Months Old; 1st Pregnancy; 1½ Days Pregnant.—The corpora lutea appear to be in the height of their development. The lutein cells composing them are rounded and suffer very little mutual pressure, being separated by fair intervals in many cases. Between them is a loose mass of fibrous connective tissue. The interstitial cells on the other hand, lie in dense masses between the corpora lutea. They are arranged in parallel strands in the manner already noted. Certain interstitial cells that become separated from the general mass are found to be rounded and of almost the same size as the lutein cells of the corpora lutea.

One is struck by the great resemblance of the interstitial and lutein cells (Plate VII, Figs. 29 and 30), a resemblance that practically amounts to identity aside from the matter of size, which difference can, in large part, be attributed to the factor of external pressure. The description of the interstitial cells of the 6-months' virgin practically applies to the interstitial cells of this pregnant rabbit and to the lutein cells of the corpora lutea as well.

Ovaries of Older Pregnant Rabbits.—The ovaries of a number of animals in various stages of pregnancy were examined. In the older of these animals the lutein and interstitial cells are apparently indistinguishable in the deeper-lying regions where the cells are the oldest. In the most central zones they are found to be undergoing a process of hyaline degeneration, the cell limits becoming indistinct, the cytoplasm ragged, and the nucleus very faint. Finally the innermost regions are found to contain the shrivelled remains of these cells.

Corpora lutea and masses of interstitial cells are successively forming at the periphery and disintegrating in the interior of the ovary.

Atretic follicles were found in adult pregnant females of various ages.

V. DISCUSSION OF RESULTS.

1. Indifferent Stage.—The genital ridge first appears as an area of thickened peritoneum and underlying mesenchyme (stroma), extending the entire length of the mesonephros, and situated on the ventromedian face of that organ. The tissues composing it are in no wise different from those forming the remainder of the investment of the mesonephros. In section, the peritoneum is found to be separated from the stroma by a more or less distinct basement membrane formed by the interlacing of protoplasmic fibrils proceeding from the nuclei of peritoneal and stroma cells (Plate I, Figs. 2 and 3).

The cells composing the peritoneum and stroma tissues are almost wholly without evident boundaries. Only here and there does one find scattered cells with distinct cell boundaries, centrosphere, centrosome, large nucleus, and clear cytoplasm—the so-called primitive sex cells. They occur in all parts of the genital ridge but are most numerous in that region in which the sex gland will form.

The distinction between peritoneum and stroma is not based upon any essential difference in the character of their component cells at this early period, but is based upon their arrangement, the nuclei of the peritoneum being arranged with their long axes parallel to one another and perpendicular to the basement membrane, while those of the stroma tissue lie with their long axes usually parallel to the basement membrane of the peritoneum which is very faint at certain points where active division of the peritoneal cells is taking place. This is due to the fact that peritoneal nuclei are being crowded through the membrane by mutual pressure, caused by their rapid multiplication.

In the 10 cm. stage, a regional differentiation begins to appear in the genital ridge. This is marked by the formation of numerous crowded peritoneal invaginations (Plate I, Fig. 4) in the middle third; less numerous and deeper invaginations in the anterior third; and the almost total lack of them in the posterior third. The regions from front to rear, as thus marked off, are the rudiments of the rete, sex gland and mesenteric ridge, respectively.

These invaginations are caused by a progressive multiplication of the peritoneal nuclei. Although the first formation of these cords is a true process of invagination, further growth is centrifugal, the peritoneum

moving outward, and at the same time adding to the cords already laid down, by a continuation of the invagination process.

These cords are truly of a tubular nature, a lumen, though not present, being conditioned by the arrangement of the cells. Transverse sections of these incipient tubules show a bounding membrana propria which is continuous with the basement membrane of the peritoneum. Inside of this is a single layer of peritoneal cells with their bases attached to the membrana propria, while their apices meet at a common central point—the rudiment of the future lumen.

The invaginations are much fewer in the rete region than in the sex gland rudiment, and also differ from those of the latter region in the fact that they penetrate through the stroma to the walls of the Malpighian corpuscles (Plate II, Fig. 6), from which the rudimentary sex cords are separated by a layer of stroma. The limit between the rete and sex gland rudiments may be roughly placed at a point opposite the 12th glomerulus, in the rabbit, and opposite the 20th, in the pig; however, the sex gland rudiment slightly overlaps the rete region; hence the impossibility of drawing a sharp limit between the two.

There has been a great diversity of opinion in regard to the origin of the sex cords and rete tubules. Practically all writers except Egli, Janosik, Coert and von Möller have derived the rete tubules from the Malpighian corpuscles. The above named, considered them as products of the peritoneum covering the mesonephros. There has been greater unanimity in regard to the derivation of the sex cords. We shall not enter into detail upon this subject, but shall simply point out the fact that Waldeyer, 70 and 02, Kölliker, 98, Balfour, 78, Rouget, 79, and others hold that the sex cords arise from the Malpighian corpuscles, and that they receive primitive sex cells which migrate to them from the peritoneum.

According to Mihalkovics, 85, the cells of the sex cords arise from the germinal epithelium, not through direct invagination but in an indirect manner, through infiltration of the stroma by peritoneal cells which later become segregated to form strands.

Schulin, 81, and Coert, 98, hold a somewhat different view, namely that the entire sex gland is formed from a homogeneous mass of cells blastema) derived from the peritoneum. This view differs from that of Mihalkovics, 85, in that the latter does not consider the stroma to be derived from the peritoneum, while Coert considers such a peritoneal origin of the stroma to be very probable, and extends this idea to explain the formation of the rete tubules and the sex cords as well.

The continued growth of the sex cords at their bases and the accom-

panying outward movement of the peritoneum results in a thickening of the genital ridge in the sex gland region. This becomes more and more pronounced until the sex gland appears hemispherical in transverse section, later appearing as a disc attached to the mesonephros by a relatively slender bridge—the mesentery. It gives the impression of having become constricted from the surface of the mesonephros. This appearance is, however, delusive, as the mesentery is in reality slightly broader than was the base of the rudimentary sex gland.

The cells composing the indifferent sex gland (Plates III and IV, Figs. 9 and 11) may be classed under three heads: (1) Primitive sex cells; (2) syncytial cells with small nuclei; (3) syncytial cells with nuclei of various sizes.

The primitive sex cells, already described, are found chiefly in the sex cords, although they occur sparingly in the connective tissue of the mesentery. They divide infrequently by mitosis throughout these early stages.

The nuclei of cells of class (2) stain deeply and are often attenuated. They form the albuginea and occur in the stroma, sex cords and, to a limited extent, in the peritoneum.

Cells of the third class occur together with those of the second class, which they resemble in that they are without definite boundaries. Their nuclei are larger and usually stain less deeply, showing all gradations between those of the primitive sex cells and the small, deeply-staining syncytial cells of class (2). It is almost certain that both forms originate from these cells of intermediate character of which the peritoneum is almost exclusively formed.

In the basal portions of the sex cords at the time when the latter are being separated from the peritoneum, there is a direct transition of the nuclei of class (3) into connective tissue nuclei of class (2), which forms the mesentery and the albuginea (Plate III, Fig. 9). Certain small nuclei of the sex cords resemble these connective tissue nuclei in a most striking manner and probably arise by a similar process of differentiation. These last named belong to the germinative cells of the seminiferous tubules and to the follicular cells of the medullary cords.

The albuginea and mesentery of the sex glands are derived from the peritoneum of regions immediately dorsal and ventral to the sex gland (Plate II, Fig. 7). The cells of these mesentery rudiments proliferate rapidly throughout the early stages, causing a rapid growth of the mesentery. Here and there primitive sex cells are formed in these regions and are carried down into the mesentery with the connective tissue in the midst of which they lie.

As stated above, the albuginea is formed by the transformation of the cells occupying the basal parts of the sex cords, into connective tissue elements, which are liberated by the rupture of the membrana propria encasing them (Plate III, Fig. 9). In this manner the sex cords become separated from the peritoneum and undergo further growth and differentiation independent of that layer.

The formation of the mesentery and albuginea and the separation of the sex cords from the peritoneum are far more clearly shown in the pig than in the rabbit, yet I have been able to verify these processes throughout in the latter animal. Coert, 98, has come to essentially the same conclusions, but is cautious in expressing himself in regard to these points, as he well may be, because of the difficulty of following these processes in the rabbit, upon which form he worked.

The separation of the sex cords from the peritoneum takes place at a slightly earlier period in the female than in the male. Coert, 98, has laid considerable stress upon this fact in the case of the rabbit. However it is not of primary importance in the pig. In the latter animal, it takes place in embryos of 1.6 to 1.7 cm. in length.

As previously stated, the rete tubules are serially homologous with the sex cords, differing from them at this stage chiefly in the fact that they are less numerous, being isolated from one another by considerable intervals, filled in with connective tissue. The portion of the genital region occupied by the rete tubules becomes elevated to such a degree as to be quite evident in gross dissections. Coert considers the rete tubules of the rabbit to arise from a mass of unorganized rete blastema by a process of differentiation which slowly progresses inward from the periphery. According to him, this differentiation process is not completed until after birth. I found these tubules to be distinct and clearly limited in the rabbit embryo of 16 days. This difference between our results may have been due to a difference of technique. Coert used Kleinenberg's picro-sulphuric solution as a fixing agent, while my material was fixed in Flemming's fluid followed by Heidenhain's iron hæmatoxylin stain.

Primitive sex cells are present in the rete tubules from the first, but are uncommon, the great mass of cells being similar in character to those of the peritoneum from which they arose. This similarity applies not only to the absence of cell limits in the rete cells of the pig and rabbit, but in the former animal, to the size and staining reaction of the nuclei as well. In the rabbit, these nuclei stain more deeply and are slightly smaller than are the nuclei of the peritoneum and of the germinative and follicular cells of the sex cords. As will be seen in a discussion of later stages these differences tend to disappear.

The rete tubules extend in a posterior direction, their bases remaining attached to the peritoneum for a long time after the sex cords have become completely separated from it. These rete tubules penetrate quite deep into the stroma, reaching the walls of the Malpighian corpuscles, to which they are often so closely approximated as to give the appearance of arising from them. The glomeruli underlying this rete region comprise those from the 6th to the 20th, inclusive, in the pig, and from the 6th to the 12th in the rabbit.

Since the rete remains indifferent in character long after the ovary and testis have become differentiated from one another, a common description will suffice to make clear its development in both male and female, up to a relatively late stage. Primitive sex cells begin to form anew from the syncytial cells of the rete tubules in the pig embryo of 3 cm. length. When the embryo has reached 4 cm. length, the rete tubules break away from the peritoneum along the posterior three-quarters of the length of the rete region. I was unable to determine whether this process is similar to that by which the sex cords become separated from the peritoneum. In any case it takes place at a much later period as above shown.

At this stage and a little earlier, evaginations arise from the capsules of Bowman of the Malpighian corpuscles at points close to the mass of rete tubules (Plate V, Fig. 15). Their number varies from one to three. In fact many Malpighian corpuscles give off no evaginations at all, although they arise in close proximity to the rete tubules. Similar evaginations occur in the rabbit embryo of 16 and 17 days, where they were first observed by Coert, 98. I am inclined to ascribe to these a morphological significance, yet they are of no particular functional importance, because a union of the rete tubules with the capsules of Bowman is, in very many cases, established by the former coming in direct contact with the latter.

It is interesting to note that branches from the rete tubules grow out to meet the tips of the evaginations from the Malpighian corpuscles. The cells from these two sources assume similar characters and are later indistinguishable from one another. Such later stages are very deceptive, having no doubt given rise to the incorrect view that the rete tubules arise from the Malpighian corpuscles.

The rete tubules branch and anastomose in their course, behaving much like the sex cords in this regard. The tubules of the anterior end of the rete mass remain in connection with the peritoneum throughout later stages while posterior to this point they are separated from it by a considerable interval and are united to form a cylindrical mass, the posterior end of which projects into the anterior end of the sex gland.

In the rabbit, the conditions are essentially similar yet by no means so clearly shown as in the pig. Difficulties in the study of these processes in the rabbit are caused by the compactness of the tissues, the smallness of the component cells, and the indistinctness of the limits of the rete tubules.

2. Sexual Differentiation.—It now remains to follow the ovary and testis separately as they diverge in the process of further development. Both are homologous, in that they have originated from an indifferent rudiment in which a considerable complexity of structure has become evident before it is possible to distinguish sexual differentiation.

Unmistakable differences between ovary and testis can be discerned in the 2.5 cm. embryo of the pig, less-marked differences being evident in the embryo of 1.8 cm. length. A clear distinction between ovary and testis is observable in the rabbit embryo of 14½ days' age.

Previous to the period of sex differentiation, the sex gland has taken definite form, having become constricted off from the mesonephros, to which it remains attached by the relatively narrow mesentery. A transverse section shows it to be composed of the following tissues: (1) the peritoneum, or germinal epithelium as it has been generally termed, especially in the case of the ovary; (2) the albuginea, a term usually applied to the subperitoneal connective tissue of the testis, but equally applicable to the same zone in the ovary; (3) the sex cords; (4) the interstitial stroma; (5) the distal ends of certain rete tubules that have grown from the rete region into the anterior end of the sex gland.

The prime features of sex differentiation are shown in the 2.5 cm. pig embryo. The fundamental points are the further development of the sex cords in the testis to form the seminiferous tubules and the development of the peritoneum in the ovary to form the cords of Pflüger. These two sets of cords having a similar origin, but one which is successive in point of time, are the structures in which the functional sex products form. On the other hand, the sex cords of the ovary cease in their growth and become the medullary cords—assuming the character of the cords of Pflüger. In the testis the peritoneum ceases to develop and becomes flattened—finally almost disappearing in later stages.

The albuginea layer is far thinner and more compact in the testis than in the ovary. This is due to the fact that in the former it is much more closely crowded against the peritoneum by the seminiferous tubules than by the medullary cords in the case of the ovary.

The peritoneum, cords of Pflüger and medullary cords of the ovary, together with the peritoneum and seminiferous tubules of the testis,

contain numerous globules of fat resulting from a process of fatty degeneration in these structures. Loisel, 00 and 02, found the spherules of fat to occur throughout the rudimentary sex gland of the 98-hour chick embryo, and in the 5-day embryo of the California quail, and in sparrow and guinea-pig embryos as well. According to him the primitive sex cells lose them when they become spermatogonia, while certain germinative and Sertollian cells contain fat globules up to the end of embryonic life, when they disappear, to later reappear at the time of puberty and at successive periods of sexual activity. There is thus a periodicity in their formation at least in the sparrow, upon which form the greater part of Loisel's work was done. He shows that the interstitial cells are filled with fat globules at the same time that the cells of the seminiferous tubules contain them, hence there is an interrelation between the two sets of cells. Interstitial cells are rare if not non-existent in the testes of adult birds, although they occur in great numbers in the testes of adult mammals.

He interprets the fat spherules described above as secretion products and not as the products of degeneration. It seems to me unsafe to hazard an opinion upon the physiological aspects of this process, yet it certainly does result in the destruction, both immediate and remote, of a large number of cells. It would hardly seem that in the present state of our knowledge, Loisel is justified in his assertion that the bright plumage assumed by birds during the breeding season is due to any trophic stimulus imparted by this fatty substance. Coincident with this process of fatty degeneration in these structures, certain cells of the stroma suffer extensive modification, their cytoplasm becoming granular, acquiring a centrosome, centrosphere and definite cell limits. The nuclei of these cells also enlarge and become spherical. These *interstitial* cells are very numerous in the testis, but quite rare in the ovary. In both sex glands, they divide by mitosis.

Plato, 97, Coert, 98, and Limon, 02, are unanimous in agreeing that the interstitial cells arise from the stroma in both ovary and testis.

3. Further Development of the Sex Glands. A. Testis.—The peritoneum becomes less and less important in later stages, finally forming a broken and almost vestigial covering of the sex gland. The albuginea becomes thicker and more compact, but need be given little further attention as its development is a very simple process.

We have still to consider the development of the following elements:

1. Seminiferous tubules.
2. Rete tubules.
3. Interstitial cells and stroma.

1. *Seminiferous Tubules.*—The seminiferous tubules of the 2.5 cm. pig embryo are rather distinctly limited by a membrana propria, still exterior to which is a thin layer of small connective tissue cells forming a capsular investment. The cells of the tubules are of two general classes—germinative cells and primitive sex cells, between which classes are found all intermediate forms. The germinative cells are identical with those classed above under groups 2 and 3, in fact the conditions are not altered in this stage. All classes of cells are dividing by mitosis while many of the germinative cells appear to be undergoing amitotic division.

Conditions in every regard similar to those outlined above, hold good in the rabbit material, the corresponding period being about the 16th day of embryonic life.

Transition forms connecting the germinative cells with the sex cells occur in the pig embryos between 2.5 cm. and 13 cm. length (see Plate IV, Fig. 14), later stages showing no transition forms. The rabbit material being more extensive, shows the condition of the cells of the seminiferous tubules up to the period of sexual maturity. Intermediate forms of cells are found to connect these two types in all stages from the 16-day embryo up to the stage 8 days after birth inclusive. Testes of the 40-day rabbit show absolutely no connecting links, the two classes of cells being there found in their purity. The germinative cells occur in a single layer with their bases attached to the membrana propria, while the primitive sex cells—spermatogonia—lie in the more axial portions of the seminiferous tubules.

A striking feature of the seminiferous tubules is their tendency to branch and anastomose (Plate IV, Fig. 12), such tendencies manifesting themselves in the very earliest stages, when the sex cords are first laid down.

As has been previously shown, the rete tubules are pushed into the anterior end of the sex gland. In the pig, their tips project into an axial space left between the inner tips of the radially arranged seminiferous tubules, while in the rabbit, the mesentery is broader than in the pig; hence there is left a space at the base of the testis, for the occupancy of the rete tubules.

2. *Rete Tubules.*—In the testis of the pig, the rete tubules remain within the hilum until a period between the 8.5 cm. and 10 cm. stages, during which time they grow rapidly down the axial space, almost reaching the distal end. It is at this period that the rete tubules begin to acquire a lumen and also to send out branches—tubuli recti—to the inner ends of the seminiferous tubules. As many as four of these

tubuli recti were seen to arise from a single rete tubule, being apparently called forth wherever needed. A distinction between the tubuli recti and the seminiferous tubules can be readily drawn from several criteria. The chief difference lies in the greater diameter, lack of lumen, and far greater number of sex cells of the seminiferous tubules as contrasted with the fact that the rete tubules are narrower by half, possess a lumen, and contain very few primitive sex cells (Plate V, Fig. 18). The two structures resemble one another in the character of their component cells, the germinative cells of the seminiferous tubules being practically identical with the epithelial cells of the rete tubules, and the primitive sex cells of both structures showing an exact correspondence. This homology is also seen in the fact that both structures are limited by membrana propria and capsular connective tissue investments formed in the same manner in each.

This process of the extension of the rete tubules takes place in the rabbit 3 days after birth. Later, the seminiferous tubules grow about the eccentrically placed mass of rete tubules in such a manner as to enclose it. Repeated anastomosis of the rete tubules results in the union of their lumina to form a large cavernous, irregular space imperfectly divided by the walls of the component tubules. The nuclei of the rete cells still have the general characters of those belonging to the germinative cells of the seminiferous tubules; but are far more elongated by lateral compression. Primitive sex cells are found in the rete tubules of the rabbit 24 days after birth, but are not present in those of the 140-day rabbit; hence it is safe to conclude that the sex cells of the rete testis are not functional.

3. *Interstitial Cells.*—In the pig, the interstitial cells are found to multiply by mitosis from the time of their first appearance up to the stage of the 7.5 cm. embryo, and in the rabbit testis as late as 8 days after birth. There may be new interstitial cells formed between the period of their first appearance and sexual maturity, but this seems highly improbable, no evidences of such having been seen. They begin to degenerate in the 15 cm. pig embryo, and in the rabbit 24 days after birth. This process of degeneration first manifests itself by a shrinkage of the cytoplasm. In the process of development of these interstitiitial cells, their cytoplasm becomes filled with fat globules that have a tendency to run together (Plate IV, Fig. 13). At the same time, the centrosphere becomes clearer and more sharply defined from the surrounding cytoplasm. Plato, **97**, does not represent the centrospheres in his figures of the interstitial cells of the cat, rabbit, steer, horse and other forms studied by him.

B. Ovary.—The tissues to be considered in this organ are as follows:

1. Cords of Pflüger and peritoneum.
2. Medullary cords.
3. Rete tubules.
4. Interstitial cells and stroma.

1. *Cords of Pflüger, and Peritoneum.*—The cords of Pflüger, were seen to arise in the 2.5 cm. pig embryo as columns of cells growing into the stroma from the peritoneum. During later stages, they lengthen by centrifugal growth, cell multiplication taking place largely at their points of attachment to the peritoneum. One can find all stages in the development of the oögonia (see Plate VI, Fig. 22) from the stage when they are indistinguishable from the other cells of the peritoneum from which they originate, to that in which more mature forms of oöcytes are found in the deeper-lying portions of the cords of Pflüger. There is a gradual transition in these cells; the degree of maturity corresponding with the distance from the surface of the ovary. In the rabbit, certain small nuclei of cells without cell boundaries divide by amitosis (Plate VI, Fig. 22). These cells of the cords of Pflüger correspond to the germinative cells of the seminiferous tubules. In the ovary, they are destined to form the granulosa cells, although it might well remain an open question whether some of these amitotically dividing cells do not also transform into sex cells.

In certain of the later stages of the pig (13 and 15 cm. embryos), tubular cords of Pflüger make their appearance (Text Fig. 3). They extend from the peritoneum for some distance into the medullary substance. By the development of the cells forming these peritoneal tubules they become transformed into solid cords containing primitive sex cells and other elements similar to the peritoneal cells of which these invaginations were originally composed. The latter are destined, in part at least, to form the granulosa. In the manner above described, these hollow tubules become transformed into solid cords of Pflüger, in every way homologous with the cords laid down at an earlier period of development. In still later stages, the cords of Pflüger are found to have widened, branched, and anastomosed to such an extent as to form an almost unbroken cortical zone, through which plates of connective tissue extend in a radial direction, marking out the original limits of the cords. Their inner ends are broken up to form nests of cells which become surrounded by layers of the invading stroma. In this manner are formed the follicles with their connective tissue theca, the oöcyte and granulosa cells being derived from the cords of Pflüger.

Kölliker, **98**, Mihalkovics, **85**, Rouget, **79**, Bühler, **94**, hold the view

that the granulosa cells are derived from the medullary cords. None of the above named authors subjected this question to a critical study of numerous stages in any species of animal; but studied isolated and more or less mature stages. Winiwarter, 00, Balfour, 78, Coert, 98, Nagel, 99, and many others hold, on the contrary, that the granulosa cells arise from the cords of Pflüger.

2. *Medullary Cords.*—The medullary cords which we found to be at a standstill in development at the time of their separation from the peritoneum develop into structures in all regards similar to the cords of Pflüger. Although homologous with the seminiferous tubules, they are distinctly female in character.

There is a constant degeneration of follicles in these medullary cords and in the deeper portions of the cortex as well. This results in the complete destruction of the sex cells in the former before the follicles have developed far enough to possess more than a single layer of granulosa cells. The few such young follicles found in the medullary cords and inner portions of the cords of Pflüger of the 13 cm. embryo, are found to have disappeared in the 15 cm. stage, leaving small clumps of more or less elongated granulosa cells enclosed in the membrana propria and connective tissue investment that was previously formed about them. These clumps remain throughout later stages, the persistent granulosa cells taking on more or less the appearance of connective tissue.

The fate of the medullary cords is quite similar in the rabbit. It will be more explicitly dealt with in connection with the rete tubules. Suffice it to say that the sex cells never pass beyond the stage of synapsis characteristic of young oocytes in a certain early stage of development, the few simple follicles that make their appearance being destined to degenerate as in the pig.

3. *Rete Tubules.*—The subject of the rete tubules of the ovary is one of the most interesting of the whole account. As previously stated, they contain primitive sex cells during the early stages in the development of both male and female. These are present in both the extra- and intra-ovarian portions. In that part of the rete lying within the sex gland, they increase in number during the 4 cm. and 5 cm. stages of the pig, becoming much more numerous than in that portion lying within the mesonephros. The proximity of any given portion of the rete tissue to the sex gland appears to condition the relative number of primitive sex cells found in it. The rete tubules of the ovary are at all times devoid of a lumen, and the intra-ovarian portions take on more and more the appearance of the medullary cords and cords of Pflüger. This similarity becomes very evident in the 13 cm. stage, at which period

the intra-ovarian portions of the rete are almost wholly composed of primitive sex cells and of smaller cells in every regard identical in kind with the follicular cells. Strands of stroma tissue serve to separate the rete tubules from one another.

Later stages show the intra-ovarian portion of the rete tissue to become constricted off from the extra-ovarian (mesonephric portion). The primitive sex cells in the former continue to develop until in the 18 cm. embryo there are found typical follicles (Plate VI, Fig. 20), each with its oöcyte and a single layer of granulosa cells. These oögonia and oöcytes are short-lived, however, being already in process of degeneration, resulting in their total destruction before the 20 cm. stage, where all traces of the sex cells have disappeared from the rete tissue, both in the ovary and in the mesonephros. The only trace of rete tissue found in the ovary at this time consists of clumps of connective tissue and elongated modified granulosa cells. These resemble the vestiges remaining after the degeneration of the medullary cords and of the inner follicles of the cords of Pflüger.

In the rabbit, the process is not so striking, the sex cells having disappeared from the rete tissue in the 17-day embryo, long before there has been any trace of follicle formation. The rete tissue is bunched at the anterior end of the ovary in contact with the medullary cords. Coert, 98, states that the rete extends by no means so far distally in the ovary as it does in the testis. I have also observed this fact in the rabbit, while in the pig it is very marked as already shown. The close resemblance between the cells of the rete and medullary cords makes it difficult and, in later stages, impossible to distinguish between them. The only criterion is the presence in the latter of scattered sex cells, these being absent from the rete tubules after the 17th day of embryonic life. This distinction, however, is quite unreliable.

The subsequent history of the mass of tissue formed by the union of the rete and medullary cords is an uneventful one. After the primitive sex cells of the medullary cords degenerate in the young rabbit, 17 days after birth, the rete and medullary cords are seen as slender strands, lying in the dense stroma between the lymph spaces of the medullary portion of the ovary (Plate VII, Fig. 26). Their nuclei often become columnar through the pressure exerted upon them. These rete-medullary cord rudiments have now reached a period of quiescence in which they are remarkably persistent, remaining until after the animal has passed the stage of puberty. In both pig and rabbit, they persist as vestigial structures, playing absolutely no further rôle in the development of the sex gland.

4. *Interstitial Cells and Stroma.*—The first generation of interstitial cells in the pig ovary appears in the 2.5 cm. embryo. They divide by mitosis, but are on the whole short-lived, disappearing in the stage of 4 cm. The stroma consists of fibrous connective tissue filling in all the space between the remaining structures, and forming a very important element of the ovary. No interstitial cells are found in the rabbit ovary until the stage of 45 days after birth, when a few cells are to be found, which can unmistakably be assigned to this class. Their presence is associated with the degeneration of certain follicles in which a theca interna has developed from the stroma investment. Such a theca interna is not formed until the follicle has acquired about three layers of granulosa cells and the rudiment of a follicular cavity. Their development can be best understood in the ovary of the 85-day rabbit. Fully-formed follicles at this stage are seen to be surrounded by a connective tissue investment which consists of an inner layer of modified cells—theca interna—and an outer layer of ordinary connective tissue cells. All transition forms between these two kinds exist (Plate VII, Fig. 26), showing that the cells of the theca interna have originated from the general stroma by a process of transformation in which the cell body becomes cylindrical instead of fibrillar, the cytoplasm becomes clearer, the nucleus larger and spherical, and a centrosome makes its appearance. In reality, the theca interna is separated from the follicle by a thin layer (follicular capsule) of attenuated connective tissue cells which send fibres in a diagonal direction through the theca interna to the theca externa. This arrangement has been previously described by a number of authors, Paladino, 87, Clarke, 98, and Rabl, 98.

Very many of these follicles are degenerating at this stage. As soon as the granulosa cells have begun to degenerate by chromatolysis, those of the theca interna begin to enlarge slightly. A few cells from the innermost fibrous layer (follicular capsule) and from the theca interna break through the basement membrane and enter the cavity of the follicle. The theca interna derivatives undergo fatty degeneration, eventually disappearing together with the granulosa cells. The only elements that ultimately persist in the mass of degenerating cells enclosed by the follicular capsule are the thin connective tissue cells that have become separated from the capsule. These remain unaltered after all the cells that have migrated from the theca interna have disappeared by fatty degeneration and the granulosa cells by chromatolysis.

During the degeneration of the cells enclosed by the follicular capsule, the cells forming the latter join to form a thick densely-staining membrane which contracts and thickens as degeneration proceeds. It

persists after all the granulosa and inner theca cells and even the ovum have entirely disappeared. It shows a more or less fibrous structure in these later stages, finally disappearing without leaving a trace. The above described closing-in of the follicular capsule stretches the connective tissue strands joining the capsule with the theca externa. In this manner, the cells of the intermediate theca interna are arranged in radiating columns. These develop into the interstitial cells to be described later. This view has already been advanced by Schottlaender, 91, Clarke, 98, Plato, 97, Limon, 02, and a number of others.

At a stage immediately succeeding the disappearance of granulosa cells and ovum, the nuclei of the still undeveloped interstitial cells become amœboid and then undergo a rapid process of amitotic division (Plate VII, Fig. 27). This is not described in any of the literature, although Dr. Frank R. Lillie and Dr. C. M. Child of this laboratory both inform me that they have noted the same phenomenon.

Van der Stricht, 01, finds that in the formation of the corpora lutea of the bat (Vespertilio) the cells of the theca interna having taken on the form of lutein cells, divide by mitosis for a short period, after which division ceases entirely. Sobotta, 96, also has found scattered mitotic figures in the theca interna of the rabbit after discharge of the ovum, although he does not ascribe to this layer the formation of the lutein cells. Rabl, 98, finds them to divide before the beginning of atresia, not after that process has set in.

After this process of amitotic division is completed the interstitial cells rapidly increase in size and finally develop into the mature form in which the cytoplasmic body is voluminous, clearly bounded, and stuffed full of fat globules; the nucleus is enlarged and rounded; and a well-defined centrosphere and centrosome have appeared. These interstitial cells are similar to the lutein cells of the corpora lutea in all regards save size. Even this criterion is not a safe one by which to distinguish the two sets of elements. Certain interstitial cells of the ovary of a 6-months' old virgin, having become separated from the mass and lying free in the loose stroma, are found to have enlarged to the dimensions of the smaller lutein cells of the corpora lutea.

Although the work of Sobotta, 96, Honoré, 00, and others has led them to assert that the lutein cells of the corpora lutea originate solely from the granulosa cells of the discharged follicle, there are a large number of workers who hold the view that they originate solely from the cells of the theca interna. Among such authors may be mentioned Clarke, 98, Van Beneden, 80, and Kölliker, 98. Van der Stricht, 01, and Schulin, 81, consider them to arise from both sources. This question

does not properly come within the limits of this work, yet I cannot refrain from pointing out the very close resemblance between the interstitial and lutein cells (Plate VII, Figs. 29 and 30). It seems quite improbable that two groups of cells, almost identical as these are, could have arisen from such diverse elements as the connective tissue cells of the theca interna, on the one hand, and the granulosa cells on the other.

Successive generations of lutein and interstitial cells push the earlier-formed groups of cells toward the center of the ovary, where they undergo hyalin degeneration. De Sinety, **77**, finds in the human subject that the number of atretic follicles is greater during pregnancy than at other times. I cannot substantiate this; but am inclined to consider pregnancy to make no difference in their number in the rabbit. This view I can support by a number of ovaries, taken from immature rabbits, mature virgins, immature virgins, pregnant animals and one taken from a rabbit that had borne young but had been isolated for three months.

4. Primitive Sex Cells.—The primitive sex cells occur from the earliest stages studied (pig, 6 mm. length; rabbit, 13-day embryo) on through all later stages of development. Similar cells have been found in the earliest stages of the Elasmobranchs by Beard, **00, 02, 03**, Rabl, **98**, Woods, **02**, and in the Teleost by Eigenmann, **91**. I have myself found the large yolk-filled primitive sex cells of these authors in turtle embryos (Trionyx) and may say that I am now at work upon this subject. It is too early to give results, but it may be stated with certainty that these cells occur in the embryo of 3 mm. length and are seen to be apparently migrating from the entoderm through the splanchnopleuric mesoderm to the point where the latter joins the somatopleuric mesoderm. It is here that the sex glands are to form. This observation corresponds with that of Beard, **03**.

The nuclei of such cells are larger than those of the entoderm and mesoderm among which they lie, but resemble them at this stage in the fact that they show very little chromatin material as contrasted with the very pronounced chromatin network which they show in later stages (1.5 cm. embryo). In this stage the large yolk spherules are found to be breaking up into small granules which remain in one or two clumps in the cytoplasm. These cells at this stage show a very marked resemblance to the primitive sex cells of the pig and rabbit. In fact I have no hesitation in identifying them as their homologues. In the pig and rabbit they are found not only in the genital ridge, but outside of it as well, in the earlier stages. So far as my own work goes, I have found them in the mesentery of the alimentary canal. Eigenmann, **91**, found

them even in the brain region of the young Teleost (Micrometrus). There seems to be no doubt of their sexual character, because they are present in such great numbers in the sex glands, and have all the characteristics of those cells (spermatogonia and oögonia) from which the sex products eventually form.

It has been shown in the pig and rabbit, however, that these sex cells, appearing in the indifferent stages, do not contribute to the formation of functional sex products in the ovary. The same is probably true of the testis, it being at least certain that the great bulk of the spermatogonia are formed from the germinative cells—cells derived originally from the peritoneum and maintaining, first, their indifferent character at least so far as our technique is able to show. Schönfeld, **01**, and Loisel, **00**, have shown, the former in the case of mammals, the latter in birds, that spermatogonia and Sertollian cells arise during adult life from these germinative cells (indifferent cells of Schönfeld). What interpretation shall we then put upon the so-called primitive sex cells (primitive ova, Ureier, Urkeimzellen, etc.)? I consider them to be spermatogonia in the testis and oögonia in the ovary. They have almost reached that degree of specialization at which we might call them oöcytes and spermatocytes. The fact that they are still found in process of mitotic divisions excludes them from these latter classes of cells. They should more properly be termed spermatogonia and oögonia of the second order, in accordance with the use of these terms by Loisel in the case of birds and by many authors who have written upon the subject of the sex cells of invertebrates.

These primitive sex cells found in the early stages of embryonic life have, then, undergone a process of precocious development; but for some reason, this process is not carried beyond a certain point. The stimuli or favorable conditions that brought about the formation of these secondary spermatogonia in regions outside of the sex gland, may be later present only in the sex gland itself, or indeed, only in certain parts of it. The influence exerted by the sex gland in bringing about the development of the sex cells is beautifully illustrated by the process above described by which egg follicles and spermatogonia develop in large numbers in the rete tubules lying within the sex gland, while they develop sparingly in those parts lying within the mesonephros. In such cases as we have seen, the degree of development and number of sex cells forming in any given region of the rete tissue is dependent upon the proximity of that region to the sex gland. It must be clearly understood that I do *not* deny the possibility of an early specialization and segregation of the sex cells as claimed by Nussbaum, **80**, Rabl, **96**,

Beard, 00, 02, 03, Eigenmann, 91, Woods, 02, and others; but I do assert that the proof of such an assertion has not yet been furnished. The development of the sex cells must be followed from the earliest embryonic stages to the period of sexual maturity, before one can prove that the cells under consideration are the only ones that give rise to the sex products, or that they give rise to them at all.

5. Morphological Relationships.—The morphological significance of the sex gland structures may be expressed in terms of the biogenetic law somewhat as follows: The genital ridge represents a primitive condition in which the sex gland extended along the entire length of the mesonephros. In this sex gland, the gonads appeared in the form of tubules or vesicles opening into the body-cavity in the case of both ovary and testis. It was impossible, in this piece of work, to determine whether there is any segmental arrangement of the sex cords in the forms studied. Such, however, would most probably be the condition in the primitive type.

The evaginations from the Malpighian corpuscles no doubt represent the "segmental stränge," "genital kanäle," etc., of those authors who have worked upon the development of the sex glands in the Anamnia. In the Amphibia, for instance, they have a truly segmental arrangement, according to Hoffman, 87, Spengel, 76, Semon, 92, while Hoffman, 89, Braun, 77, Mihalkovics, 85, Semon, 87, Weldon, 85, show a like segmental arrangement to occur in the Reptilia. Much might be said in this connection, as the literature upon the subject is quite rich in suggestive facts.

According to Shreiner, there are 2 to 3 glomeruli in each somite of the pig. My own work has shown that a connection takes place between the rete tissue and those glomeruli with which it comes in contact, there may be as many as three evaginations called forth from a single glomerulus or there may be none at all. For these reasons we cannot assert a strict homology between these evaginations and the "segmental stränge" of the Anamnia, yet the former probably represent a modification of the same process, as that by which the formation of the latter are formed.

Returning to the subject of the genital ridge as a whole, it would seem fair to conclude that the sex cords which in the ancestral forms lay posterior to the present limits of the sex gland, have disappeared. The rete tubules would represent sex cords anterior to the sex gland that had retained more or less of their ancestral condition, but have become modified to meet the requirements of their function as efferent ducts for the male sex products. The union of the rete tubules with the sex

cords is more intimate in the male than in the female, owing to the fact that the connection is not, and probably never was, of functional importance in the latter.

One is struck with the fact that there is a complete, or almost complete, separation of the medullary cords of the ovary from the peritoneum at almost the same time that the seminiferous tubules of the testis break away from it. This separation takes place essentially in the same manner in both sexes. Coert, **98,** considers the medullary cords to represent a system of ducts which served in phylogenetically earlier periods, to carry the female sex products to the Wolffian duct. Waldeyer, **70,** considers them to represent vestigial seminiferous tubules arising from the mesonephros. Paladino, **87,** and Harz, **83,** confound them in part with the long rows of interstitial cells of the adult animal. One might assume that there was one stage in the phylogenetic history of the sex glands in which both medullary cords and seminiferous tubules furnished sex products that were conducted to the rete efferentia through the rete tubules. If one hold this view, he must grant that the formation of the cords of Pflüger or second generation of ovarian cords represents a return to the primitive condition in which the female sex products are again discharged into the body-cavity from the surface of the sex gland. This view would hardly seem to be a reasonable one, hence I, at least, would prefer to consider the cords of Pflüger to be mere interrupted continuations of the medullary cords. Winiwarter, **00,** holds a view very similar to the last, his well-known diagram practically expressing my own conception of the process. There is no exact correspondence in number between the cords of Pflüger and medullary cords, the former being much more numerous that the latter.

The medullary cords never assume the characteristics of seminiferous tubules.

The assumption that the separation of the medullary cords from the peritoneum is not and never was phylogenetically of functional importance, leads to many interesting questions pertaining to the influence of heredity in the transmission of sexual characters. Is it possible that developmental processes of functional importance to the testis alone must be transmitted to the female or vice versa, simply because the germ cells which have united to produce the embryo transmit tendencies to the formation of both male and female, both of which assert their power, though one be ever so feeble? In this connection it is of great interest to note that Laulainé, **86,** has found that the peritoneum of the 7 to 8 day chick testis thickens after the separation as though it were about to develop along the line of the ovary. This is merely temporary,

as it again thins out and becomes relatively unimportant in later stages. In any case, there is a remarkably close correspondence between the general processes of development of the testis and ovary.

I wish to express my deep obligations to my professor, Dr. Frank R. Lillie, for constant guidance and valuable assistance throughout the course of this work.

VI. SUMMARY AND CONCLUSIONS.

1. The sex glands and rete originate from the genital ridge composed of the thickened mesonephric peritoneum and underlying tissue, proliferated from it.

2. The testis is composed of (A) the seminiferous tubules, (B) stroma.

A. The seminiferous tubules are formed as solid invaginations of the peritoneum, which later became separated from it, and undergo subsequent growth by the activity of the component cells. These are of two kinds, (1) primitive sex cells, spermatogonia of the second order; and (2) germinative cells. Intermediate forms connect these two kinds and may be interpreted as the primitive cells from which both varieties originate. They occur up to a certain stage in development, and may possibly recur periodically in adult stages.

B. The stroma consists of (1) loose connective tissue, (2) the albuginea—formed from the cells comprising the proximal portions of the seminiferous tubules together with possible additions of other connective tissue from the stroma, (3) interstitial cells formed from the stroma. These are formed contemporaneously with the appearance of fatty degeneration in both peritoneum and seminiferous tubules.

3. The ovary is made up of homologous groups of structures.

A. The medullary cords and cords of Pflüger are both derived by invagination of the peritoneum, the former being in all regards homologous with the seminiferous tubules. The cords of Pflüger are invaginations of the peritoneum, formed after the medullary cords have become separated from it. Both medullary cords and cords of Pflüger contain oögonia and follicle cells. Follicles formed in the medullary cords are never functional and cease to form in later stages. They degenerate together with other young follicles of the inner ends of the cords of Pflüger. Both medullary cords and cords of Pflüger contain (1) primitive sex cells (oögonia); (2) follicular cells—probably homologous with the germinative cells of the seminiferous tubules; while intermediate forms of cells are found in the peripheral part of the ovary.

B. The stroma consists of (1) loose connective tissue, from which are derived the theca interna and theca externa of the follicles; (2) a zone

homologous with the albuginea, but of a loose consistency, which renders it indistinguishable from the remainder of the stroma; (3) interstitial cells homologous with those in the testis. In the pig ovary, these interstitial cells appear very sparingly, at the same time that they appear in the testis, but very soon disappear. They develop in later stages in both pig and rabbit ovary. Details of this phenomenon were studied only in the rabbit, in which animal the cells in question were first found 45 days after birth. They are formed from the cells of the theca interna in response to conditions created by the degeneration of the follicles about which they lie. Many striking points of similarity link these interstitial cells with the lutein cells of the corpora lutea. Both finally suffer hyalin degeneration in the interior of the ovary.

4. Rete tubules are formed in connection with both testis and ovary.

A. The tubules forming the rete ovarii and rete testis originate in the region of the genital ridge anterior to their respective sex glands. They are homologous with the sex cords which they also resemble in the fact that they contain primitive sex cells. In the testis they form a core surrounded more or less completely by the seminiferous tubules, and extend almost the entire length of the organ. They project but a short distance from the hilum into the ovary. In both sexes they are connected with certain glomeruli of the anterior part of the mesonephros, by means of more or less vestigial evaginations from the capsules of Bowman.

B. Each cord of the rete testis acquires a lumen and sends numerous side branches (tubuli recti) to the seminiferous tubules, from which they differ only in diameter and in the relative number of primitive sex cells contained in them. This similarity is in later stages confined to those portions of the rete tubules that lie within the testis. These finally undergo modification in form and lose their sex cells by degeneration.

C. Homologous relations exist between the rete ovarii and the medullary cords. The rete ovarii never acquire a lumen, remaining solid, like the medullary cords which they greatly resemble. So great is this resemblance in the case of the rabbit, that the two structures cannot be distinguished from one another in post-embryonic stages. The slender strands of these indistinguishable elements persist in a quiescent state in the ovary of the adult. In the pig, numerous oögonia appear in the intra-ovarian rete tubules, many of them even developing into young follicles, all of which degenerate before birth, leaving masses of follicular cells similar in all regards to the remains of the medullary cords.

Conclusions.

1. The sex cords—medullary cords and seminiferous tubules—are homologous structures formed as tubular invaginations of the peritoneum.

2. The cords of Pflüger are formed from the same source and in the same manner as the sex cords, but at a slightly later period of time.

3. The rete cords are formed at the same time as the sex cords and in the same manner.

4. The connective tissue of the sex-gland—stroma and albuginea—is derived from the peritoneum.

5. The interstitial cells of both ovary and testis are formed from connective tissue in reference to a process of degeneration occurring in the sex gland.

6. The primitive sex cells seen in the earliest stages are precociously developed oögonia or spermatogonia of the second order, similar cells developing during later stages, from apparently undifferentiated peritoneal cells.

7. The sex glands exert a specific influence, causing follicles to form in the intra-ovarian rete of the pig, and bringing about the development of spermatogonia in the intra-testicular rete of both pig and rabbit. Such sex elements are not functional, because of the fact that they suffer early degeneration.

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EXPLANATION OF PLATES.

All figures except No. 1, were outlined with the aid of a camera lucida.

al., albuginea.
c., blood corpuscle.
c. B., capsule of Bowman.
cen., centrosphere.
c. P., cord of Pflüger.
c. p., epithelial plate.
ev., evagination of capsule of Bowman.
f. c., follicular capsule.
g., glomerulus.
ger., germinative cell.
gr., granulosa cell.
i. c., interstitial cell.
m., mesonephros.
m. f., mesenteric fundament.
m. p., membrana propria.
m. r., mesenteric ridge.
nu., nucleus.
oc., oöcyte.
p., peritoneum.
p. s., primitive sex-cell.
r., rete.
r c., rete cord.
r. t., rete tubule.
s. c., sex cord.
st., stroma.
s. tu., seminiferous tubule.
s. g., sex gland.
t., testis.
t. r., tubulus rectus.
th. i., theca interna.
th. e., theca externa.

PLATE I.

FIG. 1. Reconstruction of the anterior end of the left mesonephros. Pig embryo, length 12.5 cm.

FIG. 2. Fundament of the rete ridge. Pig embryo, length 0.7 cm. Transverse section. × 893.

FIG. 3a. Fundament of sex gland. Pig embryo, length 0.7 cm. Transverse section. × 893.

FIG. 3b. Same as 3a, showing primitive sex cell in portion of peritoneum. × 893.

FIG. 4. Fundament of sex gland. Pig embryo, length 1.0 cm. Transverse section. × 893.

PLATE II.

FIG. 5. Fundament of sex gland. Pig embryo, length 1.25 cm. Transverse section. × 608.

FIG. 6. Fundament of rete. Pig embryo, length 1.4 cm. Transverse section. × 893.

FIG. 7. Fundament of sex gland and mesentery. Pig embryo, length 1.4 cm. Transverse section. × 893.

PLATE III.

FIG. 8. Fundament of sex gland (peripheral portion). Pig embryo, length 1.6 cm. Transverse section. × 608.

FIG. 9. Fundament of sex gland (peripheral portion). Pig embryo, length 1.7 cm. Transverse section. × 893.

FIG. 10. Medullary cord of ovary. Pig embryo, length 5 cm. × 893.

PLATE IV.

FIG. 11. Rete cord in rete ridge. Pig embryo, length 1.8 cm. × 893.

FIG. 12. Seminiferous tubules and stroma of testis. Pig embryo, length 1.8 cm. × 893.

FIG. 13. Seminiferous tubule and stroma of testis (transverse section). Pig embryo, length 3 cm. × 893.

FIG. 14. Seminiferous tubule of testis (longitudinal section). Pig embryo, length 7.5 cm. × 893.

PLATE V.

FIG. 15. Capsule of Bowman and rete cords. Pig embryo, length 4 cm. × 893.

FIG. 16. Capsule of Bowman and rete cords. Pig embryo, length 7.5 cm.

FIG. 17. Intra-ovarian portions of rete cords. Pig embryo, length 8.5 cm. × 893.

FIG. 18. Tubulus rectus and seminiferous tubule. Pig embryo, length 13 cm. × 893.

PLATE VI.

FIG. 19. Seminiferous tubule. Pig embryo, length 15 cm. × 893.

FIG. 20. Follicle in intra-ovarian rete. Pig embryo, length 15 cm. × 893.

FIG. 21. Rete tubule of testis. Pig embryo, length 13 cm. × 893.

FIG. 22. Portion of cortex of ovary. Rabbit embryo, 23 days. × 893.

FIG. 23. Seminiferous tubule (longitudinal section). Rabbit embryo, 21 days. × 893.

FIG. 24. Seminiferous tubule (transverse section). Rabbit, 24 days after birth. × 893.

PLATE VII.

FIG. 25. Cord of Pflüger, with lumen. Pig embryo, length 15 cm. × 893.

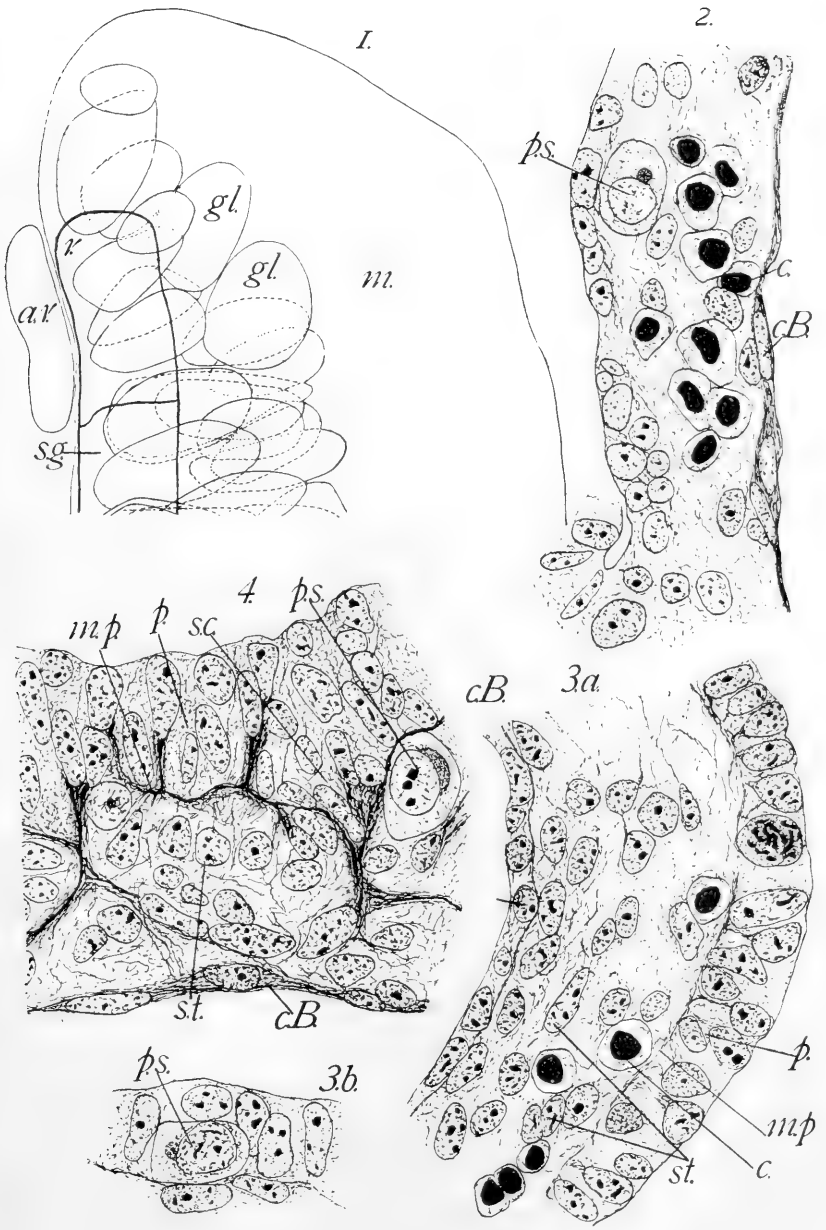
FIG. 26. Tissues of a rabbit ovary. 78 days after birth. × 893.

FIG. 27. Interstitial cells in process of amitotic division. Rabbit ovary, 93 days after birth. × 893.

FIG. 28. Interstitial cell of ovary. Virgin rabbit, 6 months old. × 893.

FIG. 29. Interstitial cell of ovary. Rabbit in 14½ day of first pregnancy. × 893.

FIG. 30. Lutein cell of corpus luteum, same animal as for Fig. 29. × 893.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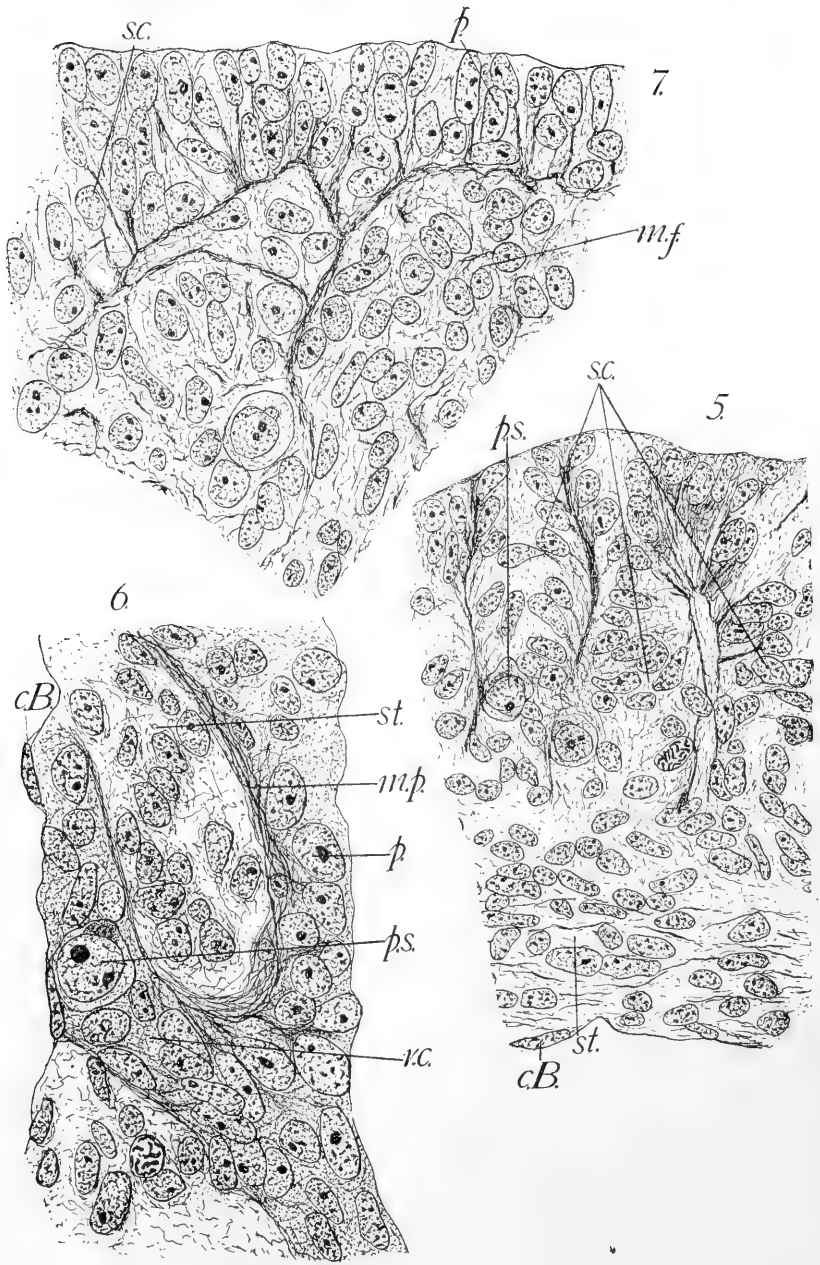
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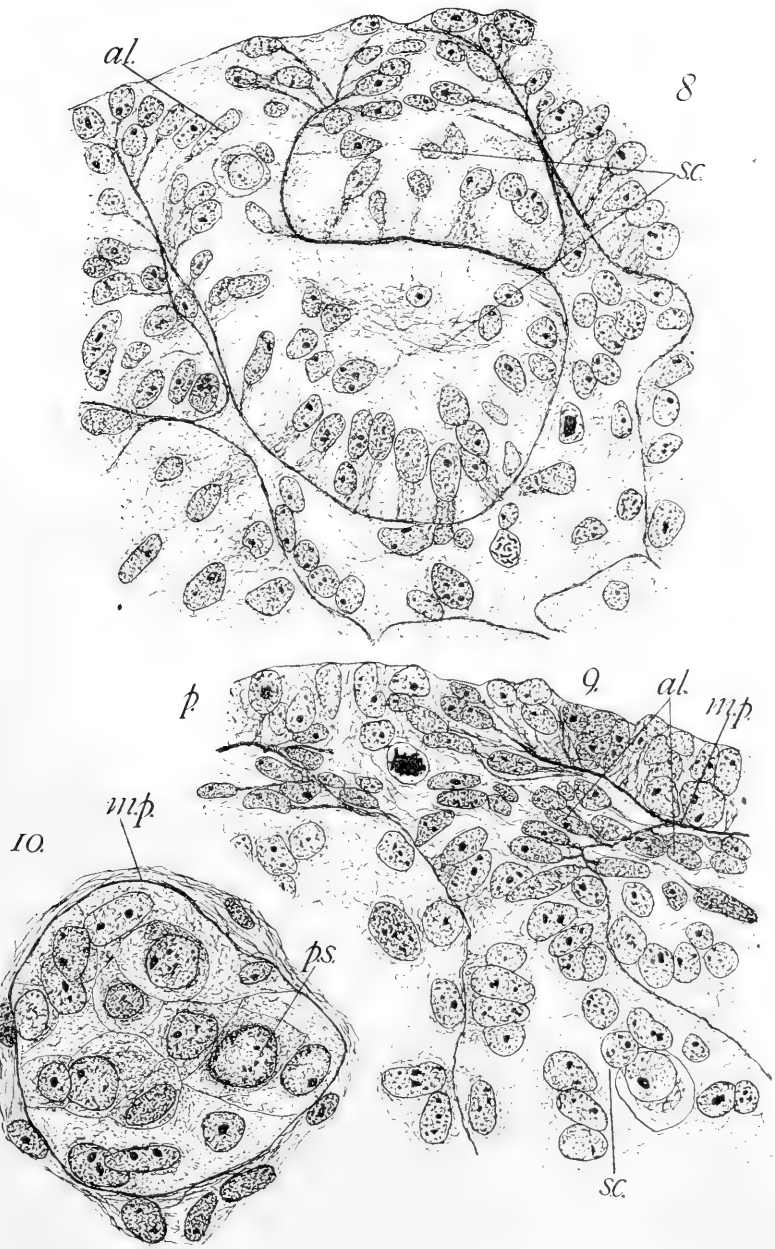
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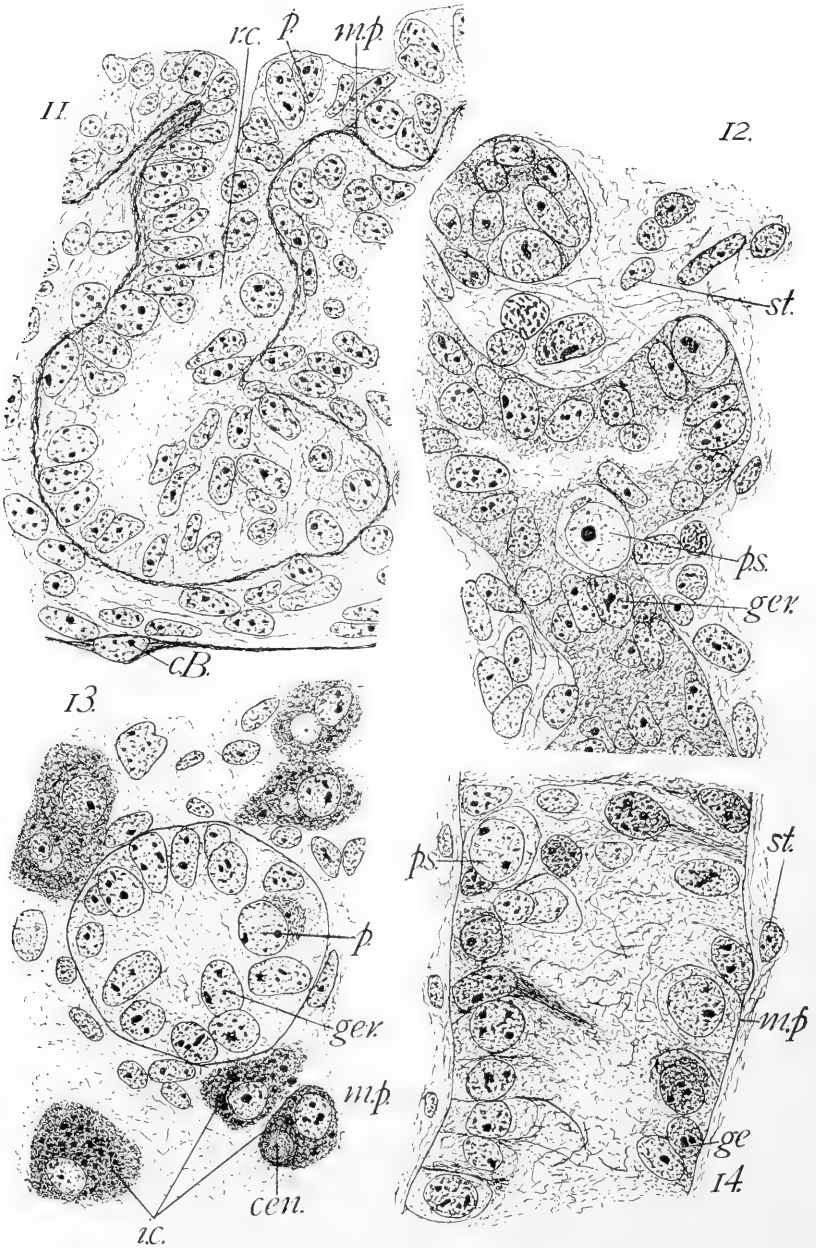
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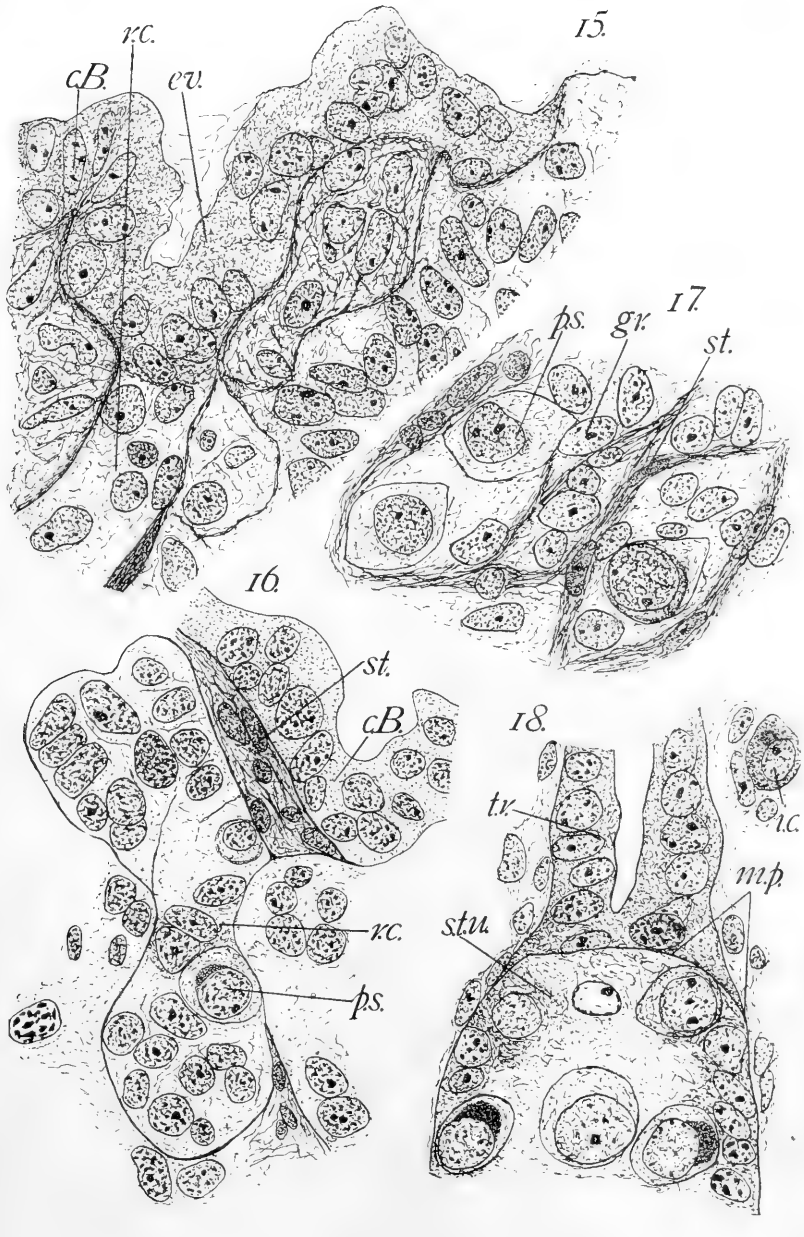
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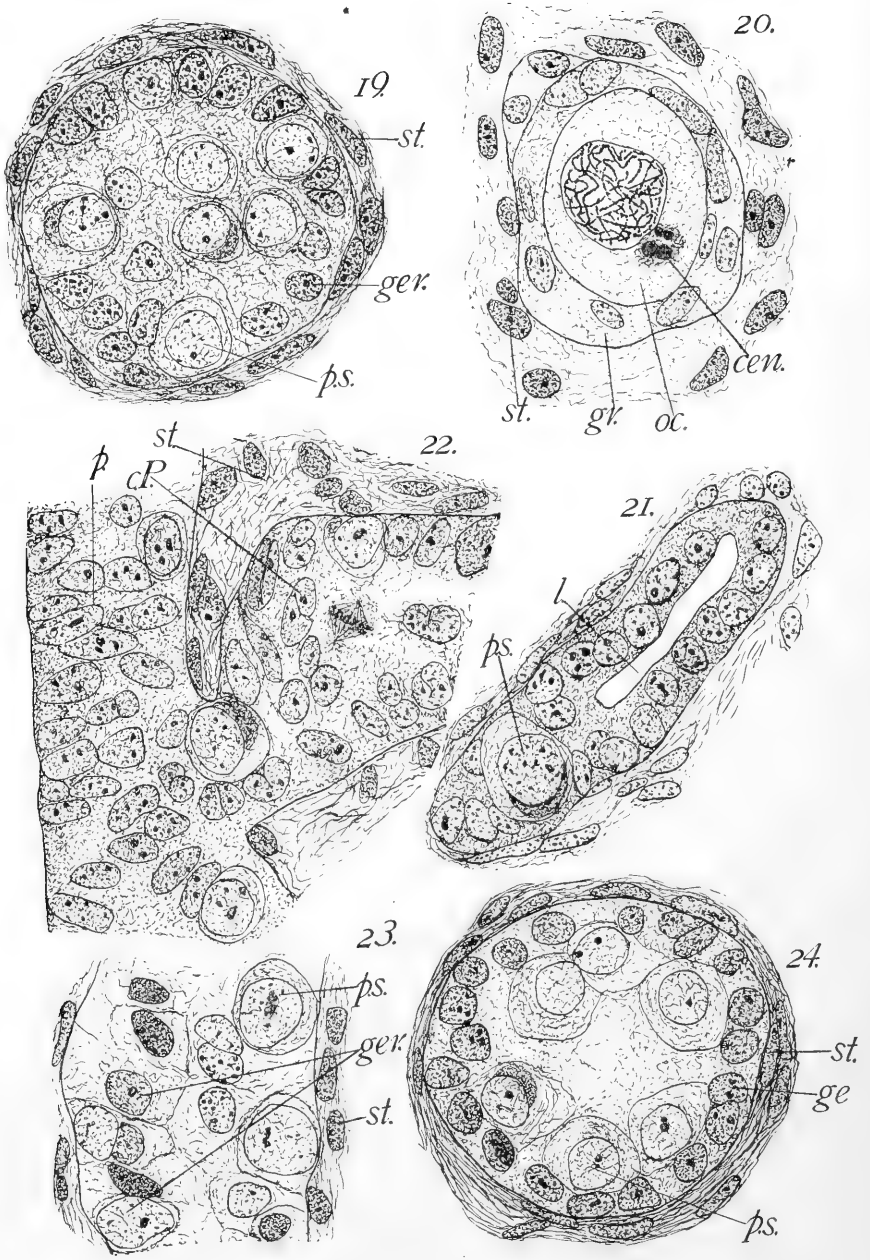
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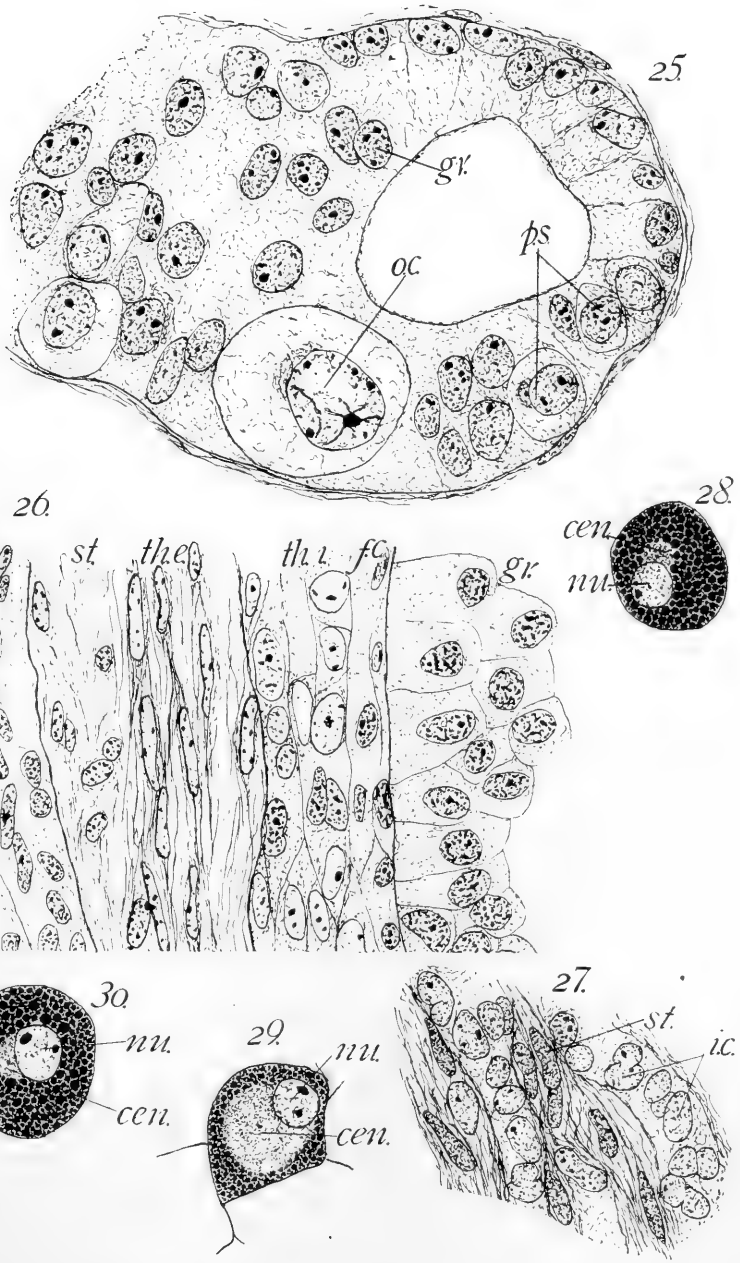
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ON THE STRUCTURE OF THE HUMAN UMBILICAL VESICLE.

BY

ARTHUR W. MEYER,

From the Anatomical Laboratory of the Johns Hopkins University.

WITH 5 TEXT FIGURES.

In the history of embryology the discovery and interpretation of the yolk sac must always remain one of the most interesting chapters. The, to us, naive speculations as to its significance, at a time when "anatomists feared to make a thorough examination of ova and preferred rather to preserve them in alcohol," lend a peculiar interest to the study of the early literature on this subject. Many of the embryologies and anatomies of that time give much attention to the yolk sac, and it is not uncommon to find several chapters devoted to the discussion.

The credit for the first description of the human yolk sac seems to lie between Hoboken and Noortwyck. Wrisberg, however, gave the first accurate description of it, in full cognizance of the fact that what he described was a yolk sac comparable to the yolk sac of birds. The latter is referred to by Wrisberg as the "vesicula erythroides" of von Pockel, unconscious of the fact that von Pockel really described the allantois and not the yolk sac, as he believed. It is possible that Noortwyck was the first to recognize the yolk sac of the human embryo. Hoboken did not recognize it, and, according to Mayer, this was left for the great Albinius who first pictured a human embryo with the umbilical vesicle *in situ*. It was this fact which caused Zinius, in his monograph, to refer to the yolk sac as "de vesicula embryonis Albiniana." Neither this designation, nor that of "vesicula alba" of Hunter, found favor, however, for both were soon displaced by the term "vesicula umbilicalis" first used by Blumenbach.

Up to 1835 the greatest diversity of opinion existed regarding the functions of the yolk sac, and many interesting theories were advanced. Oken, while recognizing the meaning of the organ and demonstrating its occurrence in several of the mammalia, promulgated the idea that the intestine arose in the vesicle itself. Kieser, in 1810, claimed to have proven that the intestine develops in the yolk sac, and that it is then slowly taken into the abdomen. Van Ruysch and Ossiander, on the contrary, took it for an hydatid and a pathological formation respec-

tively. Mayer's exhaustive monograph, which appeared in 1835, removed many of these misconceptions; though regarding its functions he says, "ueber den eigentlichen Zweck des Nabelbläschens schweben wir in gänzlicher Ungewissheit." It is interesting to note, however, that most of the early investigators ascribed nutritive and hæmatogenous functions to it.

The following study is based mainly upon eighteen normal human umbilical vesicles in the collection of Dr. Mall, to whom I am greatly indebted for the unrestricted use of his extensive collection of human embryos, and for many helpful suggestions. Besides these normal specimens, a number of pathological ones, and some taken from placentæ at birth, were examined. They were all stained in alum-carmine and imbedded in paraffin. An endeavor was made to set the imbedded vesicle so that its long diameter, which usually lay in the same direction as the remnant of the umbilical stalk, was at right angles to the microtome knife. In all cases in which this was not possible, account was taken of the fact in the study of the sections.

As the following table shows the size of the vesicles, not including those taken from placentæ at birth, varies from one to six millimeters in embryos from 11 to 110 days old:

TABLE OF EMBRYOS AND ATTACHED UMBILICAL VESICLES.

The numbered embryos in the first column refer to the cabinet of Dr. Mall.

Length of Embryo in millimeters.	Diam. of Vesicle in millimeters.	Approx. age in days.	Presence of tubules.
Embryos of the Second Week.			
Peters 0.19	0.19	..	None
von Spee.....0.37	1.08 by 1.0	12	None
No. 11.....0.80	1.00 by 1.5	13	Several
Keibel.....1.00	1.00	..	Many
v. Spee-*Gle*.....1.54	1.8 by 1.5	12	Many
Embryos of the Third Week.			
No. 12 .....2.1	1.5 by 1 by 1	13	Several
Janosik.....3.0	2.5	15	No mention
No. 76.....4.5	3.0	19	Many
No. 80.....4.5	4.0	19	Some
Embryos of the Fourth Week.			
No. 18.....7	.....	26	None
No. 2 7	7 by 4.5 by 4.5	26	Some
Embryos of the Fifth Week.			
No. 187 9	6 by 4.5 by 4.5	30	Many
No. 163... 9	5.2 by 3.7	30	Some
No. 113... ....—	.....	30	Many
No. 187..... 10	4	32	Macerated
Embryos of the Fifth and Sixth Weeks.			
No. 175.....13	3.7 by 3	36	Many
No. 167.... .....14.5	5.5 by 5.3	38	Many
No. 5.....18.5	4.7 by 4.5	42	Macerated
Embryo over Six Weeks.			
No. 22 20	5 by 2 by 2	43	Some
No. 145.....33	4.5 by 4 by 2.5	57	Few
No. 176..... 38	4.8 by 3.5 by 3.5	61	None
No. 184.....50	5 by 4 by 3.9	70	None
No. 171..... 60	6.5 by 4.2	77	None
No. X.....110	4.5 by 4	110	None

As all these measurements were made after preservation in alcohol, shrinkage must be borne in mind, although it is of no practical importance since estimations of age were not based upon them.

The vesicles are usually pyriform in shape, somewhat flattened in one diameter, and slightly roughened by protrusion and ridges below which blood islands and blood vessels usually lie. A few specimens are smooth, inflated, translucent sacs without any outward sign of blood islands or blood vessels. Others are collapsed irregularly folded and filled with calcareous-like material. There is never any regularity in the folding of the vesicle, however. Usually the folds were present while the vesicle still lay between amnion and chorion; while in some cases they were produced during the hardening and imbedding. In several of the inflated vesicles the blood vessels are plainly visible throughout their entire length and can be seen entering the umbilical stalk.

The umbilical stalk is present in the detached vesicles, as a short (5-15 mm.) stump only. It is thread-like, about .75 mm. in diameter, and never appears twisted. In cross sections the cavity of the vesicle can be traced up to the stalk, and after ending blindly a strand of characteristic entodermal cells can be traced for some distance towards the abdominal end; after which the lumen of the stalk reappears at varying intervals. This lumen, which never contains anything but a slight amount of amorphous material, is often completely occluded by the bounding entoderm.

The stalk itself is composed of three layers in the greater part of its extent. On the exterior there is a thin layer of cœlomic epithelium (mesothelium) which continues indefinitely downward over the vesicle itself. In most vesicles it stops at the upper border, but in three specimens it forms a complete outer layer. The entodermal cells which bound the lumen have all the characteristics of those lining the vesicle itself, except for a slight decrease in size. Between these two layers mesoderm is found. Nearer the body of the embryo the latter usually predominates, while it is scarcely represented at all near the upper border of the vesicle.

Besides these three layers the blood vessels form a conspicuous part of the umbilical stalk. They are not constant in number in various parts of the stalk. Sometimes three arteries and two veins are found, while in other cases one vein and two arteries are present. They can generally be distinguished by the character of their walls. The wall of the vein is formed by a single layer of very flat cells, while that of the arteries usually has an additional outer layer, composed of somewhat flattened

entodermal cells. This difference in structure, which is evident with the low power of the microscope, is found to disappear soon after the upper border of the vesicle is reached. In the structure of the walls of the blood vessels of the yolk sac itself there is never any difference as far as I am able to ascertain. The position of the vessels in both stalk and vesicle is usually well out towards the periphery, and in some cases only the cœlomic epithelium covers them.

For the microscopic structure of the youngest umbilical vesicles reference to the literature is necessary. Peters, in his monograph, gives the size of both embryo and vesicle as 0.19 mm. Unfortunately, the preservation of the umbilical vesicle of Peter's ovum was not such as to prompt a detailed description of it. We are told, however, that it is composed of entoderm and mesoderm, and in the accompanying plate (Peters, Taf. III, Fig. 33) some contents containing globules and cells are represented. In this plate the lower half of the vesicle shows no clear demarcation between mesoderm and entoderm; while in the upper half a fairly clear line of division between the two is indicated. The character of the mesodermal and entodermal cells is not given in the monograph, except that the latter are spoken of as "unscheinbaren Entodermzellen." Blood islands and blood vessels are not represented.

In an embryo of 0.37 mm. described by Graf Spee a marked advance in the structure of the umbilical vesicle exists. In this case the entoderm, which is one-layered, is composed of cubical cells, while the mesoderm is made up of irregular masses of cells with protrusions on the distal half of the vesicle, below which blood islands are found between entoderm and mesoderm. The latter is thus pushed out while the entoderm in these places is said to be more wavy, its cells of greater variety and stained more intensely.

The distal part of Graf Spee's embryo *Gle* (an embryo 1.54 mm. long) is said to be full of gaps—"äusserst lückenreich." Some of these gaps have an epithelial lining of flat cells of the nature of embryonic endothelium. Blood *Anlagen* are found in the wall of the vesicle only. In the proximal third of the latter the entoderm and mesoderm are thin and membranous, while in the distal two-thirds they are of varying thickness. The protrusions on the surface are said to be due to collections of cells between entoderm and mesoderm. It is of interest to note in this connection that Keibel states that the umbilical vesicle of an embryo 1.0 mm. long, described by him, is in every particular like that of embryo *Gle* of Graf Spee.

In the later article of Graf Spee, already referred to, he says that in embryos of three or four weeks the entoderm forms true glandular struc-

tures with small necks and large distal ends, which in embryos of nine weeks are branched and are found in all parts of the mesoderm.

It is the chief object of this study to determine, if possible, the origin and fate of these glandular structures, and to throw some light upon their possible function. So far as I have been able to learn, Graf Spee was the first to mention and to describe them, and no one else seems to have suggested any explanation of their presence. These glandular structures, which for the sake of brevity I shall call tubules, are present in the walls of nearly all vesicles taken from embryos less than two months old in the collection of Dr. Mall. The vesicles of Nos. 11 and 12 of this collection, embryos 0.8 mm. and 2.1 mm. respectively, are identical in structure. Both are in a state of good preservation, and their structure in cross section as represented in Fig. 1.

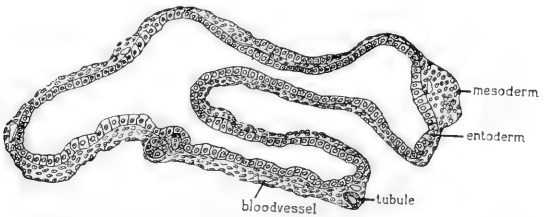

FIG. 1. Umbilical vesicle of an embryo 2.1 mm. long (No. 12). × 35.

In these vesicles a few small, short, empty, cylindrical tubules with narrow lumina surrounded by a single layer of rather large pyramidal cells, are clearly seen in the mesoderm close to the entoderm. These so-called glandular structures do not branch and can be traced through from two to five sections. *They are not in connection with the entoderm and end blindly at both ends.*

A much better though a somewhat similar picture of these tubules is found in the vesicle of embryo No. 2, an embryo seven millimeters long. Here the number of tubules is considerably greater and a direct connection between many of them and the entoderm exists (Fig. 2). In many cases the tubules end as evaginations of the entoderm and are thus in direct communication with the cavity of the vesicle. Others are indirectly connected with the entoderm by bands of entodermal cells, while still others lie isolated in the mesoderm. As shown in Fig. 3, all transitions are found from a slight evagination of the entoderm to closed tubules lying detached from the entoderm in the mesoderm. Although they can be traced through a series of fifteen to twenty-five sections *they are never seen to branch.* On the other hand the branching described by

Graf Spee is well seen in a vesicle taken from an embryo thirteen millimeters long. In such a vesicle (Fig. 4) we find an almost complete canalization of the mesoderm while the entoderm is but little changed. The

FIG. 2. Umbilical vesicle of an embryo 7 mm. long (No. 2). × 35.

tubules are much larger and longer and are formed by a layer of flat cells which often approach the cubical type. Contact of tubules is common but definite branching is infrequent. The lumina are wide and contain

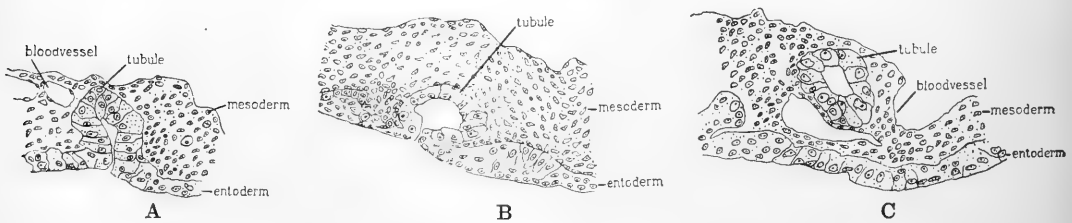

FIG. 3. Tubules from the vesicles of an embryo 7 mm. long (No. 2). × 35; (*a*) Simple evagination of entoderm—first stage; (*b*) Same, second stage; (*c*) Isolated tubule.

confused masses of amorphous material similar to that found in the cavity of many of the younger vesicles. They never seem to open directly into the cavity of the vesicle, although often the entoderm only separates their lumina from it. They are of many sizes, shapes and lengths, and lie irregularly distributed in the mesoderm. When not in contact they

often have irregular masses of entoderm between them or are separated by mesoderm. Their abundance gives a striking appearance to sections of the vesicle which is well expressed by Graf Spee as "äusserst lückenreich." It is worthy of note that the lumina of the tubules have greatly increased in diameter while the thickness of the bounding endothelium has, absolutely as well as relatively, decreased. In many cases the shape of the individual cells also has changed from cubical or pyramidal to a membranous-like layer of greatly flattened cells.

In older vesicles these tubules occur but rarely. This is usually the case in vesicles of the ninth and tenth weeks, although one vesicle taken

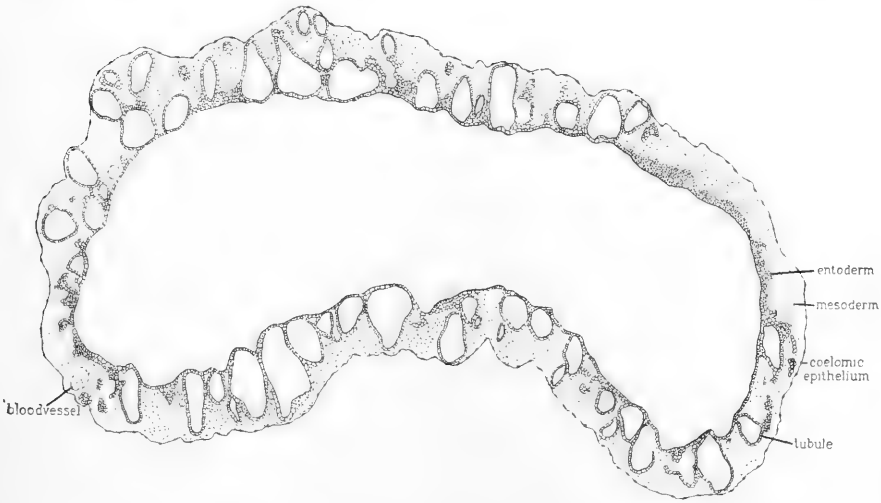

FIG. 4. Umbilical vesicle from an embryo 13 mm. long (No. 175). × 25.

from a normal embryo of the fifth week has already reached the stage of those three or four weeks older. Generally these older vesicles have a very different structure than those of four or five weeks and contain masses of calcareous matter.

It seems then that these tubules make their appearance during the second week, reach their greatest development by the fourth or fifth week and then gradually disappear by the eighth or ninth week. These stages are well represented in embryos Nos. 11 and 12; 113 and 175; and 145, 176 and 184 respectively. This conclusion is at variance with the observation of Graf Spee on embryo *Gle,* but as the widest variations as to the presence, structure and size of these tubules exist the contradiction does not seem surprising. As a rule the only constant characteristic was their direction. This was almost invariably in the direction of the

long diameter of the vesicle, for only occasionally was a tubule cut at other than a slight angle to its long diameter. Even when such was the case it could generally be accounted for by the fact that the plane of the microtome knife was not at right angles to the long diameter of the vesicle.

In spite of the large amount of material at my disposal, I am unable to reach any satisfactory conclusion as to the meaning of these tubules. Their presence is not at all a constant one. Vesicles of the same age and size often present wide divergencies of structure which are hard to reconcile. I feel justified, however, in suggesting an explanation of the manner of formation, which an examination of the material at my disposal will, I think, corroborate. Two methods of formation can be distinguished: (1) evagination of the entoderm and (2) development from irregular extensions of entoderm into the mesoderm. That the first step in the formations of many tubules is a slight evagination of the entoderm, as Graf Spee has stated, is very evident. I have found all transitions between such a stage and perfect tubules lying isolated in the mesoderm. This isolation can be readily brought about by a gradual deepening of the original evaginations accompanied by constriction and consequent fusion. This process seems to be further indicated by the occurrence of tubules which communicate with the cavity of the vesicle by their ends only, while others are closed at both ends and lie isolated in the mesoderm close to the entoderm. It seems highly probable to me that an active proliferation of the mesoderm might play a part in this separation of the tubules and their further removal to the periphery of the mesoderm.

Even if correct, however, this explanation cannot account for those tubules in whose lumina masses of unmistakable mesoderm are found. This is the case in No. 22, an embryo twenty millimeters long. In this specimen there are striking evidences of the formation of tubules by proliferation from irregular extensions of entodermal cells. Such inclusions of mesoderm might evidently result from tubule formation by invagination of the entoderm, but it is hard to find any satisfactory evidences of such a process of inclusion. That another method than that of evagination of the entoderm must have been followed, however, in the case of No. 22, is clearly indicated not only by the masses of mesoderm contained in the tubules, but especially by the fact that strands of mesoderm are found in various stages of inclusion by the entoderm. That an active proliferation of the entoderm into the mesoderm does occur, is further indicated by those specimens in which almost

the entire wall of the vesicle is composed of entoderm (Fig. 5), for in young specimens the entoderm is composed of a single well-defined layer of cells (Fig. 1).

In embryos of the seventh to tenth week the entoderm and sometimes the tubules can be found in various degrees of degeneration. This is true of Nos. 145, 176 and 184, embryos of 33, 38 and 50 mm. long, respectively. As the mesoderm is generally increased in thickness in these specimens it seems as though the degeneration of the entoderm is accompanied by a proliferation of the mesoderm. The latter at this time takes on the characteristics of a streaked fibrous connective tissue, and becomes compacted. The degeneration of the entoderm is apparent not only in the inner layer which lines the cavity of the vesicle, but is seen especially well in the groups of entodermal cells which lie scattered throughout the mesoderm. In many cases the entodermal cells are represented by granular detritus without any remnants of nuclei, while in

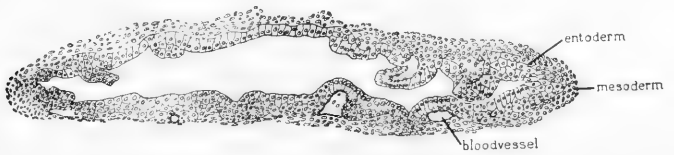

Fig. 5. Umbilical vesicle from an embryo 7 mm. long (No. 18). × 25.

other cases the cell outlines are faintly seen, and the nuclei are well preserved. Large amounts of cellular detritus can be found in the cavity of such a vesicle, and it does not seem unlikely that the cell remnants found among the calcareous contents of vesicles taken from placentæ at birth have this origin. This cellular detritus is especially well seen in Nos. 187 and 176, the cavities of which vesicles are almost completely filled with granular debris containing many large cells having the characteristics of entodermal cells. In older vesicles, those from embryos of sixty and one hundred millimeters, for example, we find, on the contrary, a condition almost identical with that found in full-term vesicles, except that the walls of the latter are more compacted and look still more as though composed of mature fibrous connective tissue.

The signs of degeneration in these older vesicles are not limited to the entoderm, however, for many of the blood vessels show marked degeneration of their walls and of the nucleated red blood cells contained within. The vessels are often pigmented and without a proper lining. The pigment reminds one strongly of blood pigment and looks very much indeed like hæmatoidin. The entire absence of vessels in the old

vesicles and their extreme vascularity in the early stages alone seem sufficient to indicate a gradual degeneration.

The walls of these vesicles, as already stated, vary greatly in thickness and in the character of the cells composing them (Figs. 2, 4, 5). Usually the greatest thickness is found at the distal end. Both entoderm and mesoderm are present in all vesicles except that of No. 187, below eight weeks of age. In these specimens the cœlomic epithelium in addition extends over the entire surface of the vesicle. This envelope is invariably composed of a single layer of very much flattened cells with elongated nuclei.

The mesoderm also presents great variations in thickness, though not in the character of its cells. These cells, though cuboidal or cylindrical in a few instances, not infrequently look like embryonic connective-tissue cells in the young vesicles, while in those of ten weeks and older it has the characteristics of fibrous connective tissue, as already noted. In these specimens it is denser, and stained more deeply near the cavity of the vesicle. The tubules and blood vessels invariably lie in the mesoderm, but are frequently surrounded by extensions or by groups of entodermal cells. In younger vesicles the blood vessels and blood islands usually cause an elevation of the mesoderm above the points where they lie.

The entoderm is composed of a single layer of cuboidal, pyramidal, and exceptionally in a small area, of cylindrical cells in vesicles of two to four weeks, but is absent in those over seven weeks of age. In a few specimens no distinct demarcation between entoderm and mesoderm can be found, though usually they are clearly defined in all the younger vesicles (Fig. 1).

A series of six umbilical vesicles taken from placentæ at birth were found almost identical in structure with the vesicles of Nos. 184, 171 and X. The walls of these vesicles are composed of a dense, wavy layer of fibrous connective tissue of varying thickness, which blends more or less with amnion and chorion. The cavity contains an irregular mass of calcareous matter among which cell remnants are plainly visible. Even those vesicles which are inflated sacs contain a small amount of calcareous matter, while those which are compressed and irregularly folded contain a firm mass of calcareous substance, which completely fills the cavity of the vesicle. Remnants of the early blood vessels or of tubules are never found nor can any recognizable remnants of the entoderm be detected. Unless, as previously suggested, the cells lying among the calcareous matter have this origin. The striking similarity between the structure

of these vesicles and those from embryos of the third month plainly shows that the condition of the vesicle as found at birth is reached early in the life of the embryo.

The occasional large size of the umbilical vesicle in full-term placentæ which contained a normal fœtus, is very remarkable. I have seen vesicles that measure fifteen by ten millimeters. Such occurrences are hard to reconcile with the supposition that the umbilical vesicle reaches its greatest development in the fourth week. Nor is it easy to see how mechanical forces can produce these large inflated vesicles. The only suggestion that occurs to me without further study of full-term vesicles, is that hypertrophy takes place at the time when the transformation of the wall of the original vesicle into fibrous connective tissue occurs.

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THE EMBRYONIC DEVELOPMENT OF THE INTERSTITIAL CELLS OF LEYDIG.

BY

R. H. WHITEHEAD, M. D.,

Professor of Anatomy, Medical Department, University of North Carolina.
From the Hull Laboratory of Anatomy, University of Chicago.

With 10 Text Figures.

The interstitial cells of Leydig furnish such a striking feature in the testis of mammalian embryos that one is surprised to find that their development has received very little study. Doubtless this is due to the fact that embryologists in their investigations of the development of the testis have had their attention focused upon the much more important subject of spermatogenesis.

These cells have been known for a long time. Leydig discovered them in 1850, and stated that they were a constant constituent of the mammalian testis. He regarded them as connective-tissue cells, and classed them with fat and pigment cells. This view was adopted by Koelliker in 1854. Boll, **69,** observed an intimate relation between them and the blood vessels, and believed that Leydig's cells composed the walls of capillaries. Von Ebner, **71,** studied them in several mammals, and concluded that they were "a peculiar form of connective tissue." F. Hofmeister, **72,** seems to have been the first to approach the problem of the nature of these cells by a study of embryonic material. Examining the testis of human embryos at four and seven months, he found that Leydig's cells constituted about two-thirds of the bulk of the gland in the embryo of four months, and only about one-tenth in that of a boy about eight years old; at puberty they were greatly increased in number, and contained much fat and pigment. He too regarded the interstitial cells as connective tissue, and thought that he could detect transition forms between them and the fixed connective-tissue cells. Waldeyer, **74,** classed them with his plasma cells, but later regarded them as perithelial. Harvey, **75,** noticed, as others had done, their resemblance to nerve cells, and advanced the view that they were derived from the sympathetic nervous system. This view, however, has been discredited by all writers on the subject.

Hansemann[1] regards it as certain that Leydig's interstitial substance belongs to the connective-tissue group, because he believed he could demonstrate an intracellular substance with Van Gieson's stain. He made the interesting observation that in the hibernating marmot no Leydig's cells were present, and that evidences of spermatogenesis were also lacking during that period; whereas in the waking animal the cells were present in such large numbers as to produce a picture resembling large-celled sarcoma. These observations, together with those of others upon fat and pigment contained in the cells, lead him to conclude that Leydig's cells constitute a distinct organ.

In 1896, Fr. Reinke[2] made the discovery of crystalloids in Leydig's cells. He found that these bodies were absent before puberty, present in large number during active sexual life, and again absent in old age.

Von Bardeleben,[3] from whose article the references to the earlier literature were taken, studied Leydig's cells in the testes of criminals, the organs being removed immediately after execution. He was impressed by the epithelial appearance of the cells, and noted that the cell-margins were not smooth, but rather serrated—the expression of intercellular bridges connecting adjacent cells. He found no intercellular substance, properly speaking, and no mitotic figures, though frequently he saw evidences of direct division. He thinks that Leydig's cells are almost identical in appearance with the Sertoli cells of the seminal tubules, and believes that they are in fact youthful forms of Sertoli cells. They are capable, he says, of passing through the walls of the tubules, there to become Sertoli cells and take the place of such as are worn out in the performance of their function. In the last analysis, according to him, Leydig's cells are epithelial in nature, and are derived from the germinal epithelium.

J. Plato[4] describes minute canals in the walls of the seminal tubules through which, he thinks, fat and pigment from the interstitial cells stream into the Sertoli cells, to be used as pabulum in spermatogenesis. To support this hypothesis he undertook a study of the development of

[1] Ueber die grossen Zwischenzellen des Hodens. Arch. f. Anat. u. Physiol., Leipzig, 1895, Physiol. Abth., p. 176.

[2] Beitraege zur Histologie des Menschen. Arch. f. mikr. Anat., Bonn, 1896, Bd. XLVII, p. 34.

[3] Beitraege zur Histologie des Hodens und zur Spermatogenese beim Menschen. Arch. f. Anat. u. Physiol., Anat. Abth., Supplement-Band, Leipzig, 1897, p. 193.

[4] Die interstitiellen Zellen des Hodens und ihre physiologische Bedeutung. Arch. f. mikr. Anat., Bonn, 1897, Bd. XLVIII, p. 280.

Leydig's cells in cat embryos,[5] using unstained sections of material fixed in Hermann's fluid. He begins his observations with the embryo of seven weeks, which, we may note, is quite a late stage. Here he finds Leydig's cells in all stages of transition to fixed connective-tissue cells, the transition proceeding from the neighborhood of the blood vessels towards the seminal tubules. He could find but one Leydig's cell containing a mitotic figure. Fat is present only in minute droplets. In the embryo at term the Leydig's cells are in close apposition with the walls of the tubules, and their nuclei are eccentric in position; drops of fat are present in the portion of the cell-body which lies opposite the nucleus. The subalbugineal layer of Leydig's cells is quite thick. In the newborn cat the subalbugineal layer of cells has almost vanished, owing to the increase in length of the tubules. Fat is wanting in many of the cells, which present, therefore, a spongy appearance. He concludes that Leydig's cells are developed from the connective tissue which accompanies the blood vessels of the testis, somewhat after the manner of typical fat cells, and regards them as trophic nurse-cells ("trophische Huelfzellen"), whose function is to pass their specific inclusions into the seminal tubules.

M. v. Lenhossék[6] confirms, in the main, the observations of Reinke as to the crystalloids. He is inclined to regard the interstitial cells as epithelial. He thinks that the presence of crystalloids in them and the absence of connective-tissue cells elsewhere in the body similar to them are decided evidence against the opinion which classes them with the connective tissues. He advances the theory that they are unused remains of the germinal epithelium, and that their function is to store up pabulum, which they give over on demand to the seminal tubules.

H. Beissner,[7] in an article intended mainly as a refutation of the opinions of Plato, calls attention to the work of M. Nussbaum in 1880. The latter held that the nests and strands of Leydig's cells were invested by a membrane similar to the wall of the seminal tubules, so that one might compare them with the Pflueger's tubules of the ovary. He suggested that they were groups of germinal epithelium which had stopped developing at an early stage—a suggestion somewhat like that of v. Lenhossék.

[5] Zur Kenntniss der Anatomie und Physiologie der Geschlechtsorgane. Arch. f. mikr. Anat., Bonn, 1897, Bd. I, p. 640.

[6] Beitraege zur Kenntniss der Zwischenzellen des Hodens. Arch. f. Anat. u. Physiol., Leipzig, 1897, Anat. Abth., p. 65.

[7] Die Zwischenzellen des Hodens und ihre Bedeutung. Arch. f. mikr. Anat., Bonn, 1898, Bd. LI, p. 794.

Among recent text-books of histology, Boehm and Davidoff state that Leydig's cells "are probably remains of the Wolffian body"; Szymonowicz says that we must assume that they are connective tissue.

Thus it appears that there are two principal views as to the histological nature of Leydig's cells. According to the one, they belong to the connective tissues (Leydig, Koelliker, v. Ebner, Hofmeister, Hansemann, Plato); according to the other, they are epithelial cells derived from the germinal epithelium (Nussbaum, v. Bardeleben, v. Lenhossék). It also appears that these views are, in the main, deductions from the study of adult conditions. It is worthy of note that the two investigators who have made a special study of the subject in mammalian embryos, Hofmeister and Plato, both conclude that Leydig's cells are derived from the interstitial tissue of the primitive testis. Their investigations, however, are incomplete, in that they were not made upon a series of embryos extending into the early stages, but upon a few isolated examples in the later stages of development.

As my work upon this subject was nearing its completion, there appeared a preliminary account of a study of the embryology of the ovary and testis by Bennet M. Allen,[8] carried out upon pig and rabbit embryos, in which the following statements are made concerning the interstitial cells: "The connective-tissue elements of ovary and testis are derived from the peritoneum. In early stages they are not distinguishable from the cells which make up the sex-cords, except that the latter are marked off from the stroma by their membrana propria . . . the albuginea is largely formed by the actual transformation of the basal parts of the sex-cords into connective-tissue elements. The interstitial cells are characterized by a large nucleus, distinct cell-boundaries, a centrosome and centrosphere, and very granular cytoplasm. They first appear in the stroma of both ovary and testis of the pig of 2.5 cm. length. They are far more numerous in the testis than in the ovary. Their appearance is coincident with that of a large number of fatty globules in the peritoneum and sex-cords. In the testis they persist for a long time. . . . In both organs they divide by mitosis. This process soon ceases in the ovary, while in the testis, on the other hand, division figures are found in the interstitial cells at a stage as late as the 7.5 cm. embryo. In the testis of the 15 cm. embryo they (the interstitial cells) have begun to degenerate. This process manifests itself in a shrinkage of the cyto-

[8] The Embryonic Development of the Ovary and Testis of the Mammalia. Biological Bulletin of the Marine Biological Laboratory, Woods Holl, Mass., Vol. V, No. 1.

plasm." "In the ovary of the 85 day rabbit they are very common, their origin from the theca interna of atretic follicles being clearly shown. This, taken in connection with the additional fact that they make their appearance in the 2.5 cm. pig embryo coincident with the fatty degeneration of the germinative cells of the seminiferous tubules and their ovarian homologues, together with that of many cells of the germinal epithelium, would lead us to conclude that cell-degeneration offers the stimulus or condition that brings about the formation of the interstitial cells."

The observations about to be described were made upon pig embryos. The material was fixed principally with Zenker's fluid, and stained with hæmatoxylin and Congo-red, iron-hæmatoxylin and Congo-red, and by Mallory's method for connective tissue. A series was fixed in Flemming's fluid, and studied either stained with iron-hæmatoxylin or unstained. Another series was used for frozen sections and staining with Sudan III. Also a few other methods were employed for special purposes.

It should be remarked at the outset that the theory that Leydig's cells are derived from the epithelium of the Wolffian body cannot obtain in the pig; for in this animal Leydig's cells appear before connection has been made between the epithelial constituents of the testis and Wolffian body. Furthermore, in the case of the pig, at least, the tubules of the rete testis grow into the Wolffian body and establish connection with the Bowman's capsules of the glomeruli, and not *vice versa*. In this connection see also J. B. MacCallum: Notes on the Wolffian Body of Higher Mammals; Amer. Jour. Anat., Balt., Vol. I, No. 3, p. 245; and the article of Allen previously referred to.

As I find myself in accord with the conclusion of Allen, that the interstitial tissue of the testis is derived from the peritoneum, meaning thereby the mesothelium of the genital ridge, I may omit the account of my study of the earlier stages, and proceed to the description of the intertubular tissue of the testis in the pig of 22 or 23 mm., a stage immediately preceding the appearance of Leydig's cells.

In the pig of this length the testis may readily be identified, as the rudiments of the tunica albuginea and the mediastinum are fairly distinct, the primitive seminal tubules are well defined, and their basement membrane is formed. The intertubular spaces in the more central portions of the gland are, on the whole, larger than those near the periphery. In the latter situation they consist mainly of capillaries derived from vessels of the albuginea, whereas in the former case they are as wide as, or even wider, than the tubules, owing to the presence in considerable

quantity of a loose cellular tissue. The constitution of this tissue is shown very well by Mallory's stain. In sections thus stained (Fig. 1) it is seen to be composed of a mixture of cells and fibrils. The cells often have little or no cytoplasm, some appearing to be mere naked nuclei; but others show a collection of cytoplasm at one pole. The nuclei are spherical or ovoid, except when closely packed together, in which case they incline to the spindle-shape. They contain much nuclear sap in which is a network of chromatin; and usually there is a

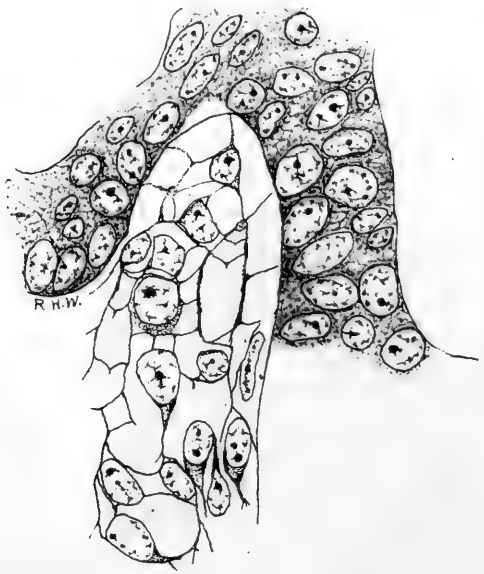

FIG. 1. *Pig 22 mm.* Shows the structure of the intertubular tissue. Mallory's connective-tissue stain. × 800.

quite distinct nucleolus. Mitotic figures are present here and there. The cells or nuclei are imbedded in a network of fibrils which take the aniline-blue of the stain. It seems clear that this tissue is a young connective-tissue syncytium in the sense of Mall.[9] In all essentials it is quite similar in structure to the deeper layers of the albuginea, with which it is continuous, and to the mesenchyme in general.

Leydig's cells were first definitely encountered in embryos 24 mm. long. In the sections they appear scattered about in the intertubular

[9] On the Development of the Connective Tissues from the Connective-Tissue Syncytium. Amer. Jour. Anat., Balt., 1902, Vol. I, No. 3.

spaces, sometimes singly, sometimes in small groups, without any very regular order as regards the other constituents of the testis, except that they are most numerous in the more central intertubular spaces; at this time there are very few, or none at all, immediately under the albuginea. Some of them show mitotic figures. They frequently arrange themselves along the basement membrane of the tubules. In size and shape they vary greatly (Fig. 2); some are spindle-shaped with the nucleus near the center of the spindle; some are oval with the nucleus in the larger end, while at the opposite end the cytoplasm tapers to a process; some are irregularly oval or spindle-shaped, while others are polygonal with eccentric nuclei. The difference in size is due principally to varying amounts of cytoplasm. Their nuclei are quite similar to those of the cells which compose the intertubular tissue of the pig of 22 mm. and stil compose the larger part of it in the pig of 24 mm. The nuclei of the Leydig's cells are perhaps larger and more spherical, and may stain more deeply, but in general they are indistinguishable from those of the other cells in the intertubular spaces. The cytoplasm is very granular, and stains well with acid dyes, so that the cells stand out very distinctly. They are markedly branched. In sections stained with hæmatoxylin and eosin the cell-margins may appear quite smooth; if Congo-red be employed as the cytoplasmic stain, some notion of the branching may be obtained, but Mallory's method for connective tissue shows the branches best (Fig. 2). The branches vary much as to size. It is difficult, frequently, to determine whether they merely interlace with one another or are in actual continuity; in some places, however, the latter relation seems clear enough to justify the conclusion that, at first, Leydig's cells form a syncytium. Figures two and three are taken from rather marked examples of this condition. In addition to thus forming syncytium, some of the processes seem to be continuous with the exoplasmic network of the fixed connective-tissue cells. Thus practically the only difference between the young "interstitial substance" of Leydig and the intertubular tissue of the preceding stage is the greater amount of cytoplasm possessed by the former; even the syncytial arrangement is retained for a short time. Hence the conclusion is drawn that Leydig's cells are derived from the cells of the intertubular tissue, which, as we have seen,

FIG. 2. *Pig 24 mm.* A group of young Leydig's cells. Mallory's connective-tissue stain. × 800.

is a mesenchymal structure differing in no essential from the mesenchyme in general.

During the next succeeding stages a number of interesting changes may be noted. The Leydig's cells undergo rapid increase both in number and size, so that they soon come to be the predominating constituent of the intertubular spaces. The fixed connective-tissue nuclei, on the other hand, become smaller and relatively much less numerous. The increase in the number of the Leydig's cells is due, in large measure, to karyokinesis, as mitoses are fairly abundant; but doubtless it is also due, in part, to the continued conversion of mesenchyme cells into Leydig's cells. These cells now begin to assume a fairly typical form; the majority of them are polygonal, and the nucleus, spherical in shape and eccentric in position, contains much chromatin and a large nucleolus. Various other shapes, however, are observed which seem to be due to mechanical conditions. Occasionally they are arranged alongside the tubules, so that the latter in cross section appear surrounded by a sheath of Leydig's cells outside of the basement membrane. Soon after they are first seen in the more central intertubular spaces they begin to make their appearance under the albuginea, where they rapidly increase, particularly large masses being found along the points of attachment of the septa. During this time also the branches begin to disappear, and soon there is no evidence of a syncytial arrangement. This change seems to occur last in the subalbugineal cells; quite a marked branching can sometimes be made out in this situation in even as late a stage as the embryo of 3.5 cm.

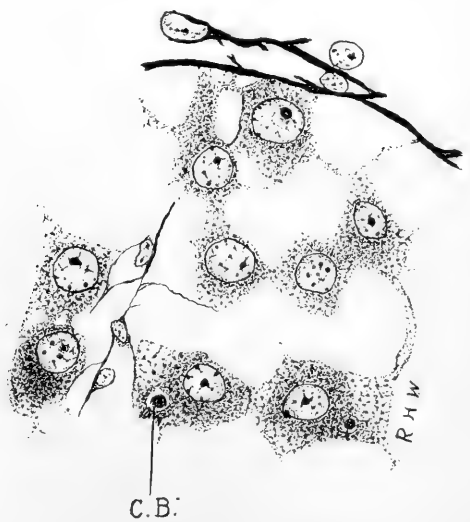

FIG. 3. *Pig 3.5 cm.* A group of Leydig's cells from just beneath the albuginea. A delicate reticulum is forming. *C.B.*, centrosphere B.,; Mallory's connective-tissue stain. × 800.

At the stage of 3.5 cm. (Fig. 3) the Leydig's cells present the greatest size to which they attain in the early embryo, and are very striking objects in preparations made by Mallory's method, which can be used so as to give a fair differential stain. They are very granular, and the

cytoplasmic network is much looser at the periphery of the cell than it is around the nucleus; the meshes of the net seem to have been distended, and the coarse granules are very apparent at the nodal points. As will be seen later, the same appearance, but in a much exaggerated degree, is found in the last stages of the embryonic development of these cells. During this period also the fixed connective-tissue cells begin to build a delicate reticulum (Fig. 3).

Following the stage shown in the embryo of 3.5 cm. there is a progressive decrease in the size of the Leydig's cells, the process affecting

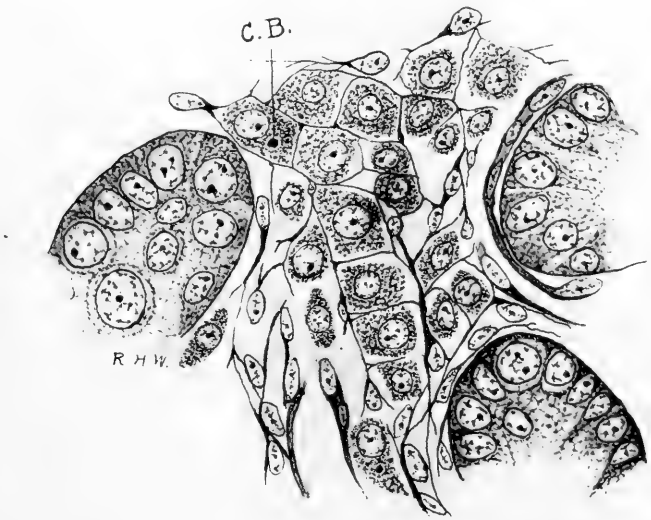

FIG. 4. *Pig 5.5 cm.* A group of tubules and an intertubular space. A quite perfect reticulum for the Leydig's cells has been formed. *C.B.*, centrosphere B.; Mallory's connective-tissue stain. × 800.

both the cell-body and the nucleus, though the change is more marked in the former (Figs. 4 and 5). There is much condensation of the cytoplasmic network, together with actual disappearance of cytoplasm. This process reaches its acme in the pig of 14 cm (Fig. 5), where many of the cells are reduced to their primitive condition of almost naked nuclei. This change was noted by Allen (loc. cit.), but the term "degeneration" employed by him scarcely seems appropriate; atrophy would doubtless be a more appropriate term. This atrophy, we shall see, is merely temporary. Very few intertubular spaces can be found which are as wide and contain as many and as large Leydig's cells as the one represented in the figure; they are very scanty also beneath the

albuginea. Between the tubuli recti, on the other hand, in which situation the intertubular spaces are much wider, they are larger and fairly numerous. A possible explanation of the atrophy of Leydig's cells is

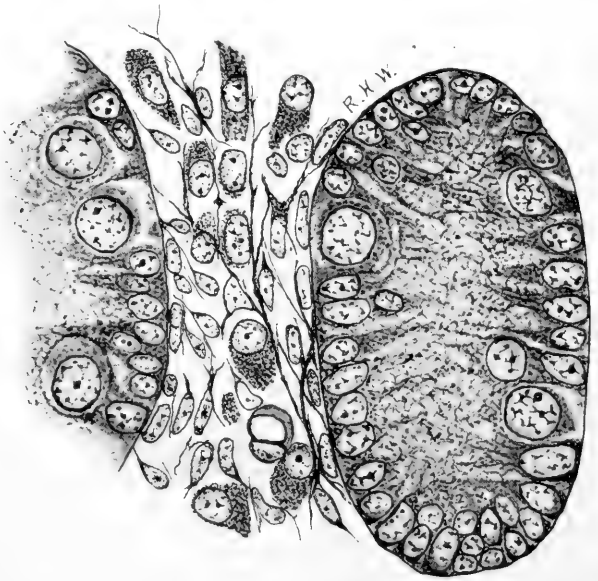

FIG. 5. *Pig 14 cm.* The tubules are much larger, the spaces and the Leydig's cells much smaller than in preceding stages. Mallory's connective-tissue stain. × 800.

suggested by a study of the growth of the seminal tubules. During the time the Leydig's cells are atrophying the tubules are growing rapidly, especially in length, and become markedly convoluted, thus reducing the width of the intertubular spaces, especially of those situated beneath the albuginea (Fig. 6). This, taken in connection with the fact that the cells of the subalbugineal region and in the narrow intertubular spaces are, for the most part, spindle-shaped, would indicate that mechanical pressure exerted by the growing tubules is a possible factor, at least, in the atrophy of the Leydig's cells. On the other hand, it might be argued that the atrophy of the Leydig's cells, by removing a physiological resistance to growth, brings about the increased growth of the tubules.

FIG. 6. *Pig 14 cm.* Tubules and intertubular spaces. Mallory's connective-tissue stain. × 70.

From the stage of 14 cm. to that of 20 cm. Leydig's cells show little

appreciable change, and seem to remain passive. After the latter length is passed, however, they enter upon a phase of activity, the most marked histological evidences of which are their great increase in size and extreme vacuolation. As this phase reaches its maximum in the embryo of 28 cm., just before term, I may pass at once to the appearances presented there. A comparison of Fig. 7 with Fig. 6 will serve to show the great change which has taken place as seen under a low power of the microscope. The intertubular spaces are now very wide and packed with large Leydig's cells, which are divided off into lobule-like groups and columns by capillaries. Under the high power (Fig. 8) the cells are polygonal with well defined cell-margins, though occasionally these may be indistinct. The nuclei are eccentric in position, and there is a

FIG. 7. *Pig 28 cm.* The intertubular spaces much wider than in figure 6 and packed with large Leydig's cells. Hæmatoxylin and Congo red. × 70.

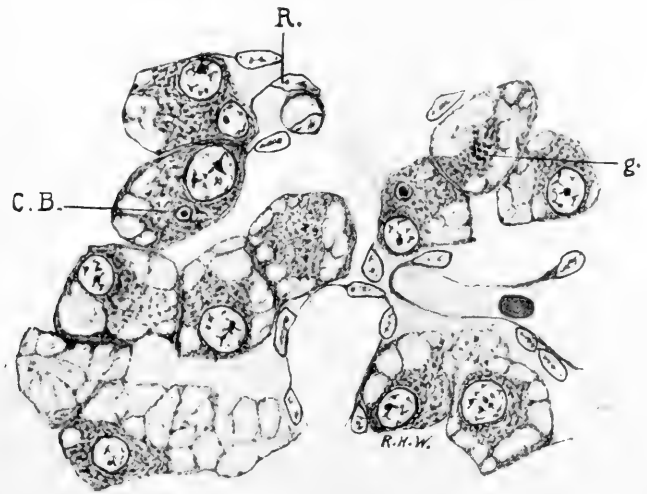

FIG. 8. *Pig 28 cm.* A small group of Leydig's cells. *g*, granules; *C.B.*, centrosphere B.; *r*, reticulum cells. Mallory's connective-tissue stain. × 800.

striking difference in the structure of the cytoplasm in different parts of the cell. In the vicinity of the nucleus it is condensed, whereas at the

periphery, especially on the side opposite to the nucleus, the cytoplasm is extremely vacuolated. Some of the vacuoles may be spherical with smooth boundaries, but many of them are irregular in shape with ragged margins, due to projecting strands of cytoplasm. The vacuoles contain no visible substance in material fixed with Zenker's fluid. Their form alone would almost warrant the conclusion that they are not fat-vacuoles, and the special tests with osmic acid and Sudan III furnish no evidence of fat in them. Cells containing acidophile granules of about the size of the eosinophile granules of certain leucocytes are of not very rare occurrence (*g* in Fig. 8); they are situated in the condensed cytoplasm of the vicinity of the nucleus. Columns of cells are often separated by wide empty spaces, the reticulum is loosened, and one gets the impression that in life the tissue must have been bathed in fluid. The histological appearances suggest a condition, not of degeneration, but rather of active metabolism; the cells which were so greatly atrophied in the pig of 20 cm. have entered here upon a phase marked by increase both of size and of physiological activity. It should be stated here that I was not able to demonstrate mitoses in the Leydig's cells of pigs longer than 7 cm., nor could I feel sure that they multiply by direct division; so that I shall have to leave open the question whether or not new Leydig's cells are formed in the later stages by cell-division. I do not think, however, that there is any doubt but that the atrophied cells found in the pig of 20 cm. are quite able to develop into the large cells of the pig at 28 cm.; for the steps of the process can easily be followed in a series of embryos. In this connection we may recall the finding of Hansemann (loc. cit.) in the marmot.

Observations on the Centrospheres.—In the early stages Leydig's cells present two structures in their cytoplasm with great regularity and constancy (Fig. 9). One of them (*C. A.* in the figure) is a large sphere, containing a small body at or near its center, from which delicate radiations proceed toward the periphery of the sphere. This periphery is formed by small granules in a row. The sphere is always situated in the immediate neighborhood of the nucleus, sometimes at an indentation in the nucleus. The second structure (*C. B.* in the figure) is also a sphere, containing a central body, but it differs from the first structure in several particulars. The sphere is smaller, but the central body is many times larger than that of the first structure. With the highest

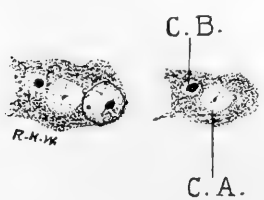

FIG. 9. *Pig 27 mm.* Two Leydig's cells. *C. B.*, centrosphere B; *C.A.*, centrosphere A; Mallory's connective-tissue stain. × 800.

power of the microscope at my command, the sphere contains no radiations, though sometimes there are a few minute grains in the clear space around the central body. The latter, as was said, is much larger than that of the first sphere; it is not homogeneous, but seems to be constituted by an aggregation of granules. Its outline frequently is circular, but often it is irregular and its periphery uneven, and its size is variable. This sphere is almost always at some distance from the nucleus, though when it alone is found in a cell, it may be near the nucleus. The central bodies of both spheres stain with iron-hæmatoxylin; also Mallory preparations show them quite clearly, and they are readily made out in unstained sections of Flemming material. The great majority of Leydig's cells seen in the early stages present one or the other of these structures, and a great many of them show both at the same time, so many, indeed, that I think both may be regarded as normal constituents. Their morphology, staining reactions and constancy make it possible that both are centrospheres, and I shall call them such. The point, however, which I should like to emphasize is that the first structure—the large sphere with the small centrosome—is not permanent, but soon disappears; it could not be found in embryos of greater length than 3 cm. The second centrosphere, however, persists, and is found in all the succeeding stages of embryonic development (Figs. 3, 4, 8); even in the atrophied cells of the pig at 14 cm. they still can be demonstrated. In the later stages, however, the centrosome often seems smaller, more homogeneous, and more regularly circular in outline (Fig. 8); the sphere is usually smaller and its wall more homogeneous in appearance, so that the whole structure somewhat resembles a vacuole with a hyaline content.

The Occurrence of Fat.—The occurrence of fat in the seminal tubules and Leydig's cells of various mammals has been noted by several observers. Allen (loc. cit.) bases a theory upon the presence of fat in the primitive seminal tubules and germinal epithelium, suggesting that fatty degeneration of these cells may furnish a "stimulus or condition which brings about the formation of the interstitial cells."

For the study of this subject I used material fixed in Flemming's solution, employing unstained sections as well as sections stained with iron-hæmatoxylin. Owing to the doubts which have been raised as to the reliability of osmic acid as a test for fat, the results thus obtained were controlled by frozen sections stained with Sudan III. In the germinal epithelium of pigs from 2 cm. to 4 cm. it was not possible to demonstrate any fat with either osmic acid or Sudan III. In all these

stages there are many cells of the germinal epithelium loaded with large granules, but as will be seen later, they certainly are not fat.

With respect to the primitive seminal tubules it was found that sections of Flemming material, stained with iron-hæmatoxylin, showed many black particles, whereas the same material unstained showed very few, or none at all, until the length of 3.5 cm. was reached. It was not until a still later stage was reached that fat could be demonstrated with Sudan III. Making allowances for imperfection of technique, it hardly seems possible that fat could have been present in such large quantity as to constitute a veritable fatty degeneration, and have escaped detection by both the osmic and the Sudan III, especially as each reagent gave good results in later stages. In the light of recent studies of fat metabolism the presence of some fat in these cells would not seem pathological. In pigs of 8 cm. fat is present in the seminal tubules in the form of quite distinct globules, and remains present in that shape through all the remaining stages of their embryonic development.

In the case of the Leydig's cells I could not positively demonstrate a fatty content in pigs under 14 cm. in length. Small globules of fat appear a little later, and are fairly abundant in the Leydig's cells of the 28 cm. embryo. In all cases they are minute droplets situated in the vicinity of the nucleus, and not in the vacuoles previously described. It may be noted here that Plato (second reference) found very little fat in the Leydig's cells of the wild boar.

The Granules of the Germinal Epithelium.—As previously stated, many of the cells which compose the germinal epithelium in pigs of the various lengths from 2 cm. to 4 cm. are loaded with large granules (Fig. 10). While they were first noticed in sections prepared by Mallory's method, they are quite distinct in all the different methods employed. In unstained sections they appear as colorless, homogeneous, glistening, more or less circular bodies. They are unaffected by agents which dissolve fat, such as ether and absolute alcohol. In preparations stained with iron-hæmatoxylin they appear intensely black. They also stain well with the aniline-blue in Mallory's method, and in general are acidophile, though they can be stained faintly by gentian violet in Weigert's method for fibrin. Occasionally a group of them is seen in a cell which has wandered down from the germinal epithelium into the albuginea;

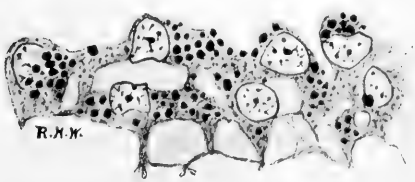

FIG. 10. *Pig 25 mm.* Peritoneal epithelium with granules. Iron hæmatoxylin and Congo red. × 800.

and once, in a pig of 3.5 cm., a small collection was seen in a Leydig's cell just beneath the albuginea. They are not yolk granules, for they do not stain with osmic acid, and no similar granules are seen in the cœlomic epithelium elsewhere. A probable explanation of their nature is furnished by the changes which occur in the germinal epithelium at this time. Its cells become vacuolated, and are soon reduced to the squamous cells which cover the tunica vaginalis. So that the granules found in the cells while these changes are going on are probably the products of a hyaline degeneration of the cytoplasm.

Summary.

The intertubular tissue of the testis of the pig embryo in stages immediately preceding the appearance of Leydig's cells is a mesenchymal structure derived from the mesothelium of the genital ridge. Histologically, it is a connective-tissue syncytium, consisting of cells and an exoplasmic network of fibrils. The cells are scarcely more than naked nuclei, though some have a small collection of cytoplasm at one pole (Fig. 1).

From the cells of this tissue Leydig's cells are developed by growth of cytoplasm. At first they are markedly branched; some of the branches are connected with the general exoplasmic network, while others unite with one another to form a network, so that the cells retain the syncytial arrangement of their ancestors (Figs. 2 and 3). They increase in number and size very rapidly, and soon lose their branches. At first they may have various sizes and shapes, but one form soon predominates. Such a typical Leydig's cell is polygonal, its cytoplasm is very granular, and its nucleus is eccentric and contains a large nucleolus. Mitotic figures can be seen in all the earlier stages.

Leydig's cells pass through two phases of growth, between which a phase of atrophy intervenes. Growth is very rapid from their appearance in the embryo 2.4 cm. long until the length of 3.5 cm. is reached. This is followed by the phase of atrophy, during which the cells return almost to their first state of nearly naked nuclei (Figs. 4 and 5). This process reaches its acme in the embryo 14 cm. long. Synchronous with it there is extensive growth of the seminal tubules, particularly in length, so that they are much convoluted, and the intertubular spaces are correspondingly narrowed (Fig. 6). In the embryo 20 cm. long the cells enter upon the second phase of growth, which attains its maximum in the pig of 28 cm., very near to term. Here the cells are enormously increased in number and size, so that they constitute the predominating

feature of the microscopic picture (Fig. 7). The nucleus is eccentric in its position; around it the cytoplasm is condensed, while at the periphery of the cell, especially at the opposite pole from the nucleus, it is extensively vacuolated (Fig. 8). Many of these vacuoles are irregular in shape with ragged margins, and none of them contain fat. The appearance of the cells suggests that they have been bathed in fluid.

In unstained sections of material fixed in Flemming's fluid no dark-brown particles were observed in the seminal tubules of pigs of less length than 3 cm.; with Sudan III no fat could be demonstrated in the tubules of pigs of less length than 8 cm., after which stage it was constantly present in the shape of globules. The germinal epithelium contains no appreciable amount of fat, the granules observed there being of an entirely different nature. We conclude, therefore, that there is no fatty degeneration of the seminal tubules and germinal epithelium in the early stages of the development of the pig's testis, and that, consequently, the hypothesis which attributes the growth of Leydig's cells to fatty degeneration in these situations is incorrect.

No fat could be demonstrated with osmic acid or Sudan III in Leydig's cells of embryos shorter than 14 cm. After this stage it was found in the shape of minute droplets situated in the cytoplasm near the nucleus, but not in the large vacuoles.

In the young Leydig's cells two structures are found, which, from their morphology and staining reactions, may be classed as centrospheres (Fig. 9). Never more than one of each kind is present in the same cell, but the same cell often contains both at the same time. The large sphere with the small centrosome is soon lost, while the small sphere with the large centrosome persists through the whole period of embryonic development, though it undergoes certain changes in the late stages.

In pigs from 2 cm. to 4 cm. long many cells of the germinal epithelium are loaded with large granules (Fig. 10). They are hyaline material, resulting from a hyaline degeneration of the cytoplasm of the cells during their conversion into the squamous cells of the tunica vaginalis.

ON THE DEVELOPMENT OF THE SUPERFICIAL LYMPHATICS IN THE SKIN OF THE PIG.[1]

BY

FLORENCE R. SABIN, M. D.,

Associate in Anatomy, Johns Hopkins University.

With 7 Text Figures.

In a previous paper[2] has been given an account of the origin of the lymphatic ducts from the veins by the budding off of blind sacs from their endothelial lining. The growth of these blind ducts toward the skin and their gradual spreading over the surface of the body was described briefly and illustrated by a composite picture. In the present communication the spreading of the superficial lymphatics will be described more in detail as well as the growth of these ducts in the different layers of the skin.

The lymph ducts bud off from the veins in four places; two in the neck, at the junction of the jugular and the subclavian veins; and two in the posterior part of the body, from the vein which enters the Wolffian body and which is formed by the union of the femoral and sciatic veins. As the Wolffian body dissappears, and its venous system is supplanted by the vena cava, this lower connection of the lymphatics with the veins is given up. From these four points of origin the lymphatics grow first along the veins toward the skin, and secondly along the aorta and its branches to the various organs. The superficial lymphatics to the skin follow the veins, the jugular in the neck and the femoral and its branches in the lower part of the body. The deep lymphatics follow the arteries; primarily the aorta making the thoracic duct, and secondarily the branches of the aorta.

[1] This paper together with one on the Development of the Lymphatic System and a part of a paper on the Development of Lymph Glands, soon to appear in this Journal, was accepted by The Association for Maintaining the American Woman's Table in the Zoölogical Station at Naples and for Promoting Scientific Research by Women.

[2] On the Origin of the Lymphatic System from the Veins and the Development of the Lymph Hearts and Thoracic Duct in the Pig. The American Journal of Anatomy, Vol. I, No. 3.

In embryo pigs below 18 mm. in length there are no lymphatics in the skin, as has been proved both by numerous negative injection experiments and by their absence in serial sections. The first sign of the lymphatic system was found in a pig 14.5 mm. long. It consisted of two small blind ducts which had budded off from the vascular endothelium at the junction of the cardinal and subclavian veins on either side of the neck. These ducts were found to grow into the neck along the anterior cardinal or jugular veins to a point midway between the ear and the scapula, and here widened into a sac. This sac, though possessing a lining of a single layer of endothelial cells without a muscle coat, I have considered to be analogous with the lymph hearts of the amphibia. From the sac the ducts grow directly outward to the skin, which they reach when the pig is 18 mm. long.

FIG. 1. The lymphatic system in the skin of a pig 2.5 cm. long. × 3.

In Figs. 1 to 5 is given a series of actual injections of the lymphatic ducts in the skin of pigs of increasing sizes. Each picture is a drawing from one actual injection, and all of the injections are practically complete except Fig. 3. That is to say, there are no lymphatics in the skin at these various stages excepting those which are shown injected. The methods of these injections are given in the paper cited above.

Fig. 1 represents the lymphatic ducts in the side of the neck of a pig 2.5 cm. long, and shows that the ducts are growing in two directions, first over the back of the head behind the ear, and secondly over the scapular region. In Fig. 2, from a pig 3 cm. long, these two tufts of lymphatics, one behind the ear and the other over the scapula, are more distinct and have increased in complexity. A new set of ducts has reached the surface at the angle of the jaw and has begun to grow out in two directions, first between the eye and the ear, and secondly in front of the eye.

Fig. 3, from a pig 3 cm. long, does not show the entire lymphatic sys-

tem of the skin of a pig of that stage, for the ducts at the angle of the jaw are not injected, nor a set of ducts which has just reached the skin over the crest of the ileum. This group of lymphatics is shown farther developed in the next figure. Fig. 3, however, does show the primary set of ducts, that is, those that grow over the back of the head and shoulder completely injected. It brings out clearly the character of

FIG. 2. The lymphatic system in the skin of a pig 3 cm. long. ×3.

the plexus, the irregularity of the ducts and the fine channels that connect neighboring wide ducts. It shows also the growing sprouts that run out in advance of the plexus to invade new areas of the skin, areas which up to this time have had no lymphatics.

In Fig. 4, from a pig 4.3 cm. long, the ducts of the primary plexus have grown to the median line in the back and anastomosed with those of the other side. The ducts over the face are well injected. The figure shows also that the lymphatics for the lower part of the body have

reached the skin at a point over the crest of the ilium. From this point the ducts radiate to the skin over the side, back and hip. From no other center of radiation for the primary lymphatics do the ducts spread out so symmetrically, so like the spokes of a wheel, as in this case. In

FIG. 3. A partial injection of the lymphatic system in the skin of a pig 3.5 cm. long. The primary group of ducts is completely injected. × 3.

the neck there are several centers of radiation, so that no one center sends out ducts in every direction.

Fig. 5, from a pig 5.5 cm. long, is the last of the series. The injection was made by two insertions of the hypodermic needle, one over the scapula with the needle opening toward the neck, and the other just below the point of radiation over the crest of the ilium. In this way one takes advantage both of the radiating direction of the ducts and of the larger

size of the primary ducts. The ease of injection in any direction shows that there are no valves at this stage, though the flow of any injection

FIG. 4. The lymphatic system in the skin of a pig 4.3 cm. long. × 3.

mass is irregular on account of the great variation in the size of the channels. In making these injections it is essential to enter the needle into the level which contains the lymphatics. As will be shown later,

FIG. 5. The lymphatic system in the skin of a pig 5.5 cm. long. × 3.

this level is the line between the subcutaneous tissue and the chorium. When the needle enters the subcutaneous tissue in pigs from 3 to 8 cm. long the injection mass spreads out in straight lines and forces a path for itself in the tissue spaces. When the needle enters the chorium the injection mass raises a bleb on the surface. In neither of these cases, that is, when the injection mass has entered the tissue spaces of the subcutaneous tissue or of the chorium in small embryos, have I ever succeeded in getting a lymphatic injection. To obtain a perfect injection without any extravasation at the point of puncture one must enter the needle at exactly the right level, that is, between the subcutaneous tissue and chorium and then inject slowly. One then sees the ducts starting out from the open slit in the injection needle. By giving a scarcely perceptible pressure on the piston of the hypodermic syringe it is possible to inject the entire side of the embryo and have the individual ducts leading from the needle stand out clearly at the end, that is, to have no extravasation. Thus it will be noted that it is necessary to puncture the ducts in order to get a lymphatic injection.

In Fig. 5 the ducts have covered the body and only the feet, a part of the head and the tail remain unsupplied. It will be noted that the ducts from the different centers in the neck have anastomosed so freely in the skin that it is not easy to see just where the primary points of radiation lie. Moreover, the ducts for the anterior part of the body have anastomosed so freely over the surface of the body with those for the posterior part that it is possible to inject into the ducts over the ilium and have the injection mass pass toward the veins in two ways; first through the ducts that come to the surface over the crest of the ilium, and secondly by an indirect course through the channels over the side of the body to the ducts in the neck.

In a pig 6.5 cm. long the spreading of the superficial lymphatics in the skin is practically completed. That is to say, ducts have been injected to the top of the head, the snout, the ears, eyelids and toes. In these areas, far from the centers of growth, the plexus of ducts is not abundant at this stage, indeed, to use one area as an example, only a few of the advance sprouts over the top of the head have actually anastomosed with the ducts of the other side. However, no area of the skin is wholly without lymphatics. In other words, the invasion of the skin by lymphatics is complete though the plexus of lymphatics in the skin is very incomplete.

To sum up: In the anterior part of the body there are three main centers from which the superficial ducts spread out; first, in the posterior part of the neck, for the ducts over the back of the head and over

the scapula; second, at the angle of the jaw, for the ducts of the face; third, in the front of the neck, for the ducts of the lower jaw, chest and fore legs. The ducts of all these systems anastomose freely in the skin. In the posterior part of the body there are two centers for the radiation of the ducts, first over the crest of the ilium for the ducts of the posterior part of the back and of the hip, and secondly in the inguinal region for the ducts that grow into the abdominal wall and down the leg. The ducts of the anterior and posterior systems anastomose freely over the body.

Having traced the spreading of the superficial lymphatics in the skin from the time the ducts first come to the surface in the neck and over the crest of the ilium to the time when they have reached the remotest parts of the body, namely, the top of the head and the tips of the toes, it remains to trace the development of these ducts in the different layers of the skin.

In pig embryos 13 mm. in length the epidermis is from two to four cells deep and is separated from the connective tissue beneath by a distinct basement membrane. The connective tissue beneath is loose or compact in different parts of the body, and is not divided into layers, so that there is no differentiation between the chorium and the underlying subcutaneous tissue. The blood capillaries in this connective tissue are large, having a width of from two to four or five times the diameter of the red blood corpuscles, which at this stage are large nucleated cells.

By the time the embryo is 15 mm. long, a stage just about the time that the lymphatics are budding off from the veins but before they have reached the skin, there are certain areas of the body, for example, over the arm bud, where the connective tissue beneath the epidermis is divided into two distinct layers, a denser layer next the epidermis and a looser meshed layer just within. The outer, denser layer is to become the chorium, and the inner, looser layer the subcutaneous tissue. The blood capillaries lie at this stage on the inner border of the chorium between it and the subcutaneous tissue. There are numerous vessels in the subcutaneous tissue but none in the true skin.

Soon the blood capillaries begin to grow outward into the chorium and give up their position along its inner border. The vessels in the subcutaneous tissue remain and become larger. As the blood capillaries advance into the true skin the lymphatic capillaries grow in just behind them, taking the position along the inner border of the chorium. In an embryo 3 cm. long the lymphatic capillaries lie in this border just internal to the blood capillaries. Fig. 6 shows the lymphatics in the skin

over the shoulder of a pig 5 cm. long. It will be noted that there is a clear differentiation between the chorium and the subcutaneous tissue. In the chorium the protoplasmic network is fine and closely meshed, while the subcutaneous tissue is more fibrillar, the tissue is more open or the spaces are larger. The lymphatics are large and lie in the border between the chorium and subcutaneous tissue. The blood capillaries are small

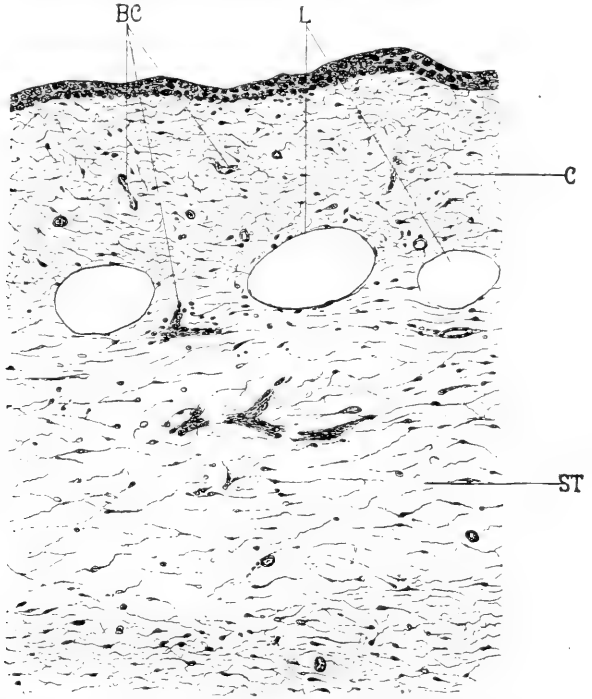

FIG. 6. Transverse section of the skin of the shoulder of a pig 5 cm. long showing the primary lymphatics. About × 110. *bc;* blood capillaries; *c,* chorium; *l,* lymphatics; *st,* subcutaneous tissue.

and lie both in the subcutaneous tissue and in the chorium. All of the vessels within the chorium are confined to its inner half.

From this time on, until the embryo is 6.5 cm. long, the lymphatics gradually spread over the entire body in a single layer of ducts. These ducts make a characteristic plexus, as shown in Fig. 5. The plexus has been described and illustrated in the paper cited above. The growth of the ducts within the plexus has been described by Ranvier[2] and by Mac-

[2] Ranvier: Comptes Rendus, 1895 and 1896. Archiv d'Anatomie, 1897.

Callum.[3] The original discovery of this method of growth was made, not by Ranvier, as I stated in a previous paper, but by Langer[4] in 1868. At the time of my first publication I had not seen Langer's paper. He published a series of papers on the lymphatic system of the frog, and in one of them on the lymph vessels in the tadpole's tail, he gives beautiful pictures of the lymphatics, with their complete lining of endothelium and with the long sprouts of endothelial cells from their walls. Some of the sprouts he shows as still solid, others partly injected. He recognized that this represents the method of growth; moreover, he states that without doubt the lymph capillaries and the blood capillaries develop in the same way—their elements being the same.

The plexus of growing lymphatics is well seen when the freshly injected skin of the embryo pig is stripped off and examined under a binocular microscope. It can thus be made out that the ducts spread out practically in one plane.

By the time the pig is 8 cm. long an injection of the lymphatics shows the primary plexus well developed. Many of the vessels are large and the plexus is wide meshed. At the same time the skin viewed under the binocular shows that there are numerous sprouts from the primary plexus which are growing outward into the chorium. These small, new sprouts do not as yet make a perfect plexus within the chorium. Sections of the skin at this stage bring out three points: First, that the ducts of the primary plexus now lie deeper in the subcutaneous tissue rather than just in the border between the subcutaneous tissue and the chorium. Secondly, that there are a few lymphatic vessels within the chorium; and, thirdly, that the blood capillaries are nearer the surface of the skin than the lymphatics.

By the time the pig is 10 or 11 cm. long the lymphatic capillaries within the chorium have become a complete plexus. Viewed under the binocular microscope, there are now two distinct layers of lymphatics, a deeper plexus with wide spaces between the ducts and a more superficial plexus of finer ducts more closely crowded together. In sections the deeper plexus is subcutaneous, while the superficial lies about the middle of the chorium. The complexity of the plexus varies greatly in different parts of the body, for example, there are many more lymphatics in the ear than in an area of skin of equal size over the back. The stages of development are given for the skin over the shoulder, the

[3] MacCallum: Arch. f. Anat. u. Phys., Anat. Abth., 1902.

[4] Langer: Die Lymphgefässe im Schwanze der Batrachier-Larven. Sitzb. d. k. Akad. d. Wissensch., I. Abth., Juli Heft, Jahrg. 1868.

development in the remoter parts, for example, in the feet, is always somewhat retarded.

While the pig is increasing from 10 to 25 cm. in length the two lymphatic plexuses, the deep or primary and the superficial or secondary, become more complicated. Valves begin to develop in the lymphatics and increase the difficulty of obtaining lymphatic injections. By the time the embryo is 16 or 18 cm. long the valves are present and prevent much backward injection. At this stage, and still more clearly in pigs between 20 and 25 cm. long, a subcutaneous injection of considerable pressure will usually enter the deep plexus of lymphatics and run centralward in the ducts of the subcutaneous tissue, but not outward into the plexus of the chorium. This is readily demonstrated by injecting into the foot-pads. If the injection is in the hind feet the fluid enters the ducts of the subcutaneous tissue and is carried to the inguinal glands; if in the fore feet, the ducts lead to the glands in the front of the neck. A subcutaneous injection then in a pig about 20 cm. long enters the deep lymphatics. The injection mass, however, often enters the chorium, not in the superficial lymphatic plexus, but rather through certain veins that run directly to the surface and spread out in a fine plexus just beneath the epidermis. These vessels are blood capillaries, as can be proved by making a venous injection. Prussian-blue was injected into the umbilical vein of a pig 22 cm. long, under a pressure of 100 mm. of mercury. The skin soon showed fine points of blue, and each point was seen to be a fine plexus of ducts just beneath the skin, the plexus spreading out from a small vein which ran to the surface. Thus, since by subcutaneous injections in these stages one usually gets a mixed injection of deep lymphatics and superficial veins, the lymphatics are best studied by complete venous injections.

Fig. 7 is a section of the skin of the ear of a pig 22 cm. long in which Prussian-blue was injected into the umbilical vein under a pressure of about 100 mm. of mercury. The veins are filled with blue granules, the capillaries with blood corpuscles, while the lymphatics are empty. The epidermis is now several layers deep, the hairs are partly developed, but there are no papillæ. There is still some differentiation between the subcutaneous tissue and the chorium, the former being more fibrillar and having wider spaces, the latter being denser and more cellular. The lymphatics are not as large as in earlier stages and they lie in two planes, a primary plexus of ducts in the subcutaneous tissue and a secondary plexus in the chorium.

To complete the study of the development of the ducts in the skin it

was necessary to find out whether the lymphatic capillaries enter the papillæ or not. The papillæ are present in the skin of the new-born pig, but the hairs make the skin so difficult to study that the papillæ are best seen in the tongue. By making a complete arterial injection and forcing the injection mass over into the veins of a pig a week old, it was easy to demonstrate the lymphatics in the subpapillary layer and

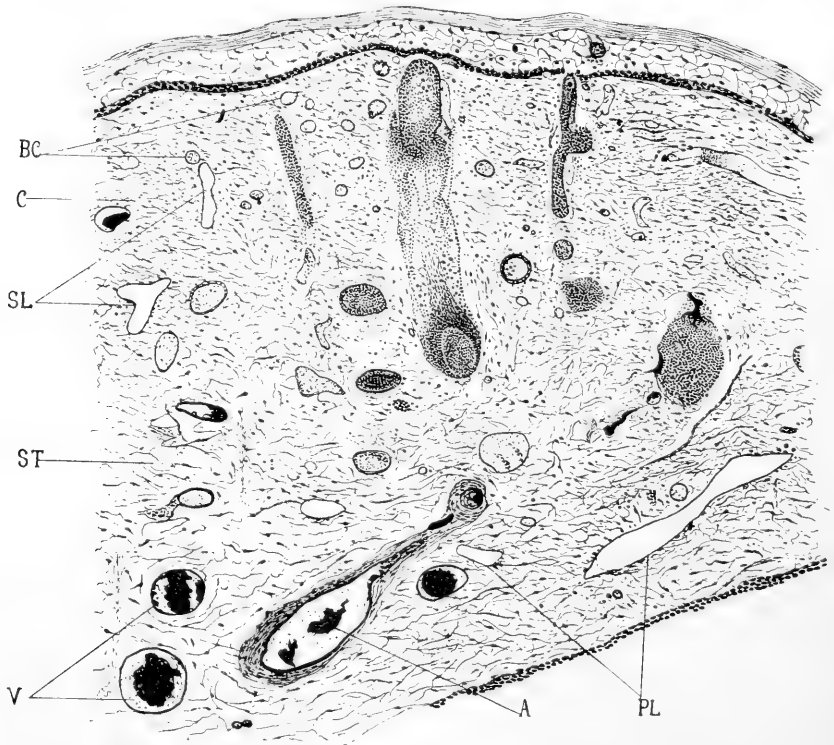

FIG. 7. Skin of the ear of a pig 22 cm. long. The veins are injected with Prussian blue represented as black. About × 95. *a*, arteries; *bc*, blood capillaries; *c*, chorium; *pl*, primary lymphatics; *sl*, secondary lymphatics; *st*, subcutaneous tissue, *v*, veins.

in the center of the larger papillæ. The smallest papillæ contain just a tuft of blood capillaries in the center, while the larger ones at the side of the tongue have a central artery which is bordered by a central lymphatic duct lined with epithelium. This makes the papillæ in the tongue analogous with the villi of the intestine as far as the central lymphatic duct is concerned.

Thus the course of the development of the lymphatics has been followed in the skin. The ducts are defined as channels with an endothe-

lial lining which bud off from previous lymphatic ducts, the original ones coming from the endothelium of the veins. The development has been traced by making injections along the lines in which the lymphatics grow to the skin. In the neck the ducts grow toward the skin along the jugular vein and come to the surface at three points; in the posterior part of the neck, at the angle of the jaw and in the front of the neck. In the posterior part of the body the ducts follow the femoral and its branches and come to the surface first over the crest of the ilium, and secondly in the inguinal region. From these points the ducts invade the skin and form a primary plexus in the subcutaneous tissue and a secondary one in the chorium. From the plexus in the chorium sprouts grow outward into the center of the papillæ. In their entire growth the lymphatics follow the blood vessels.

The lines of growth of the lymphatics to the various organs are along the course of the aorta and its branches. For example, by injecting into the edge of the wall of the aorta it is possible to inject the ducts as they are entering the heart and the lungs. The early ducts to the kidney are large and easy to obtain. By the time the pig is 4 cm. long the ducts can be injected to the stomach wall and have grown between the folds of the mesentery to the intestinal wall. Repeated injections would probably show the growth of the ducts into the different layers of the intestinal wall to their end in the central chyle vessel of the villi.

AN EXPERIMENTAL STUDY OF THE RELATION OF THE NERVOUS SYSTEM TO THE DEVELOPING MUSCULATURE IN THE EMBRYO OF THE FROG.

BY

ROSS GRANVILLE HARRISON, Ph. D., M. D.,

Associate Professor of Anatomy, The Johns Hopkins University, Baltimore

WITH 18 TEXT FIGURES.

While the rôle of the nervous system in development has been studied with increasing interest in recent years, the data are as yet of such a varied and conflicting nature as to preclude the possibility of satisfactory generalizations. The following pages are therefore offered as a contribution to one phase of this subject, in the hope that through the study of comparatively simple particular problems we may advance towards some general conclusion.[1]

The influence of the nervous system on the regeneration of lost parts has formed the subject of several experimental studies. Herbst, **99**, and **01**, has shown that in the decapod crustacea, the optic ganglion is an essential factor in determining the character of the appendage regenerated after the amputation of the eye. Morgan, **01**, has reported experiments, showing that it is the presence of the nerve cord at the cut end of a decapitated earthworm that determines the regeneration of a new head. In planarians, according to Bardeen, **02** and **03**, the stimulus to the regeneration of a new head arises from the cut surface of the central nervous system. Barfurth, **02**, and Rubin, **03**, have investigated the effect of injury to the nervous system upon the regeneration of the tail and limbs in the amphibia, concluding that the destruction of the spinal cord and brain has no deterrent effect upon the development of a new appendage. In the case of the limbs, according to the same investigators, regeneration begins normally even when the nerves running to the stump

[1] A brief account of this work was given in a paper read before the Association of American Anatomists at Washington, D. C., December 30, 1902. HARRISON, 03, On the Differentiation of Muscular Tissue When Removed from the Influence of the Nervous System. Proc. Assoc. Amer. Anat., p. IV, AMER. JOURNAL OF ANATOMY, Vol. II.

are destroyed, although later the absence of nervous influences, or perhaps the lacking function, tends to retard the processes and ultimately brings them to a standstill. Wolff, **02,** has made somewhat similar experiments upon the axolotl, and concludes that the nervous system does exercise a morphogenetic function in the regeneration of the limbs.

The data are more meagre concerning the nervous regulation of purely ontogenetic processes. Loeb, **96,** was the first to study this question experimentally; he showed that the metamorphosis takes place simultaneously in the posterior and anterior portions of the amblystoma larva even after the spinal cord has been severed. Later it was shown by Schaper, **98,** that the frog embryo develops normally after the removal of the entire brain.

A considerable mass of evidence having a bearing upon this question, has been collected from the study of acephalic and amyelic monsters. This evidence is, however, conflicting and the same facts have not always been interpreted in like manner by all investigators. Leonowa, **93,** and Fraser, **95,** have on the one hand described human fœtus in which brain and spinal cord were totally lacking, while the peripheral sensory nerves and the musculature were normally developed. On the other hand, E. H. Weber, **51,** Neumann, **01,** and others have described cases in which absence of certain portions of the central nervous system has been accompanied by total absence of musculature in the region normally supplied by the lacking nerves, although skeleton, blood vessels and even tendons were normally developed.

The discussion has centered especially around the question of the dominance of the differentiation of the voluntary musculature by the nervous system. Neumann, **01,** has given a critical *résumé* of the facts bearing upon this question. He concludes that in cases similar to those described by Leonowa and Fraser, where there is a well-developed muscular system in spite of the total absence of the brain and cord, the nervous system must have developed in the early stages of embryonic life up to a certain point, and that it did not undergo degeneration until after the differentiation of the muscular system had taken place. Thus in his effort to harmonize the seemingly conflicting observations referred to above, Neumann[2] reaches the conclusion that the physiological relations between muscle and nerve change during the course of the development of the individual as follows:

1. The first development of the muscles takes place under the influence of the nervous system and through the agency of the motor nerves, which

[2] Op. cit., p. 463.

grow from the latter into the muscle. Self-differentiation of the muscles does not take place.

2. After the muscles have arisen, their nourishment and further growth during the embryonic period takes place independently of the central nervous system; they have, so to speak, emancipated themselves from the influence of the latter.

3. Not until the post-embryonic life is reached is the dependence again established; the trophic centers of the spinal cord and brain then begin action.

Herbst, **01**, analyzes the same data, however, and maintains that it is the sensory nerves including the cells of the spinal ganglia, and not the motor nerves, that are necessary to stimulate the differentiation of the muscular substance in the embryo. Herbst finds support for this view in Wolff's observations referred to above and also in the fact that in Leonowa's case, as well as in others, the spinal ganglia and sensory nerves alone were present. Herbst and Neumann agree, nevertheless, in holding that the nervous system exerts a formative influence upon the muscular tissue. The well-known fact that a muscle undergoes atrophic changes after its nerve supply has been cut off, would, at first sight, uphold this view. The study of normal development likewise affords some evidence which might also be interpreted as lending support to it, though it does not necessarily do so. In the embryos of lower vertebrates, for instance, the connection of the motor spinal nerves with the muscle plates is established just at the time when the contractile substance begins to be laid down.[3] Again, as Nussbaum, **94** (also later publications), has shown in a series of investigations, there is a close parallel between the direction of the intramuscular ramifications of the nerve supplying a muscle and the direction of the growth of that muscle in the embryo, a view which has also been supported by Bardeen, **00**. Nusbaum, **02**, points out, however, that this correspondence might exist even though there be no dependence on the part of the muscle upon a formative stimulus.

It is clear from the foregoing that the facts are insufficient to determine even the comparatively simple relations between the nervous system and the developing musculature. The difficulty in interpreting correctly the meaning of the teratological cases, which have been the subject of so much discussion, rests upon our inability to find out the exact nature of the original lesion. The only way to control satisfactorily this factor is

[3] This apparently does not hold for all vertebrates, for, according to Bardeen, **00**, the musculature of the pig embryo is differentiated to a considerable degree before the nerves establish a connection with it.

by direct experimentation, but in devising experiments for this purpose it is necessary to formulate clearly just what is to be determined, for it is obvious from the facts already referred to, that the nervous system may possibly exert its influence in a variety of ways.

The first questions which I had in mind in beginning the present investigation were the comparatively simple ones whether a stimulus from the nervous system is necessary in order to start the differentiation of striated muscle fibers and whether the musculature is dependent upon the nervous system in its further development, including the grouping of the fibers into individual muscles. It is the first question that has of late been most freely discussed and has been answered affirmatively by Neumann and Herbst; even Rubin emphasizes the fact that of all the tissues in the regenerating limb the voluntary muscle is the most dependent upon the integrity of the nerves. While on the other hand Schaper's experiments do show that the central nervous system has no general directive action upon the development of the frog, they do not answer the first question just stated, for Schaper removed only the brain, leaving the spinal cord intact; and besides, embryos 6 mm. long with well-developed tail were used; in such embryos the motor nerve roots have already established connection with the muscle plates and the differentiation of the contractile substance is begun. The musculature at the time of experimentation in Schaper's experiments would thus fall into the second period of Neumann. To test this question it is necessary to remove the spinal cord at a period of development, before there are traces of peripheral nerve fibers or contractile substance in the musculature. A series of experiments of this kind is described below in the first section.

Another question which may to advantage be considered in connection with that of the formative influence of the nervous system, is whether the normal processes of ontogeny are regulated by functional stimuli, or to state a more particular phase of the problem, whether the normal exercise of function is a necessary factor in determining the early course of development of the musculature. While the first series of experiments may be used in this connection, the necessary mutilation of the embryo enters as a disturbing factor. This question may be best tested experimentally by causing the suspension of muscular function in developing embryos through the action of a drug. Acetone chloroform is exceedingly well adapted to this purpose. In the second section of the present paper the results of rearing embryos in solutions of this substance are given.

Description of the Experiments.

The experiments described below were made upon the embryos of Rana sylvatica, R. virescens and R. palustris, for the most part in the spring of 1902. As already emphasized, it was necessary for the purposes in view to work with embryos in which there were no traces of histological differentiation in the nervous or muscular systems. It was found on examination of serial sections of normal embryos that the oldest stage which safely fulfills this requirement is when the tail bud is just beginning to be perceptible. Sylvatica embryos (Fig. 1) are then about 3.7 mm., virescens about 2.25 mm., and palustris about 2.9 mm. in length, although, owing to the considerable variation in the size of embryos of the same species, these measurements are to be regarded merely as roughly approximate. There are absolutely no nerve fibers in the central nervous system of these embryos and there are no traces of any peripheral nerves. The tissue of the myotomes consists of rounded cells somewhat flattened on their sides. About ten somites are distinctly marked off. The rest of the axial mesoderm is unsegmented.

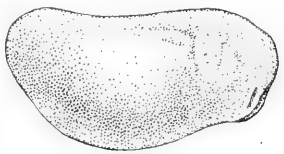

Fig. 1. Embryo of R. sylvatica. to show the stage of development used in the beginning of the experiments. × 9½.

1. On the Effect of Removal of the Spinal Cord upon the Development of the Axial Musculature.

The embryos of R. palustris are somewhat better adapted for this experiment than the other species. In the former the axis of the trunk is straight, while in embryos of R. sylvatica the dorsal curvature is marked. Virescens embryos are more difficult to operate upon on account of their smaller size.

The embryo is laid on its side in a shallow dish lined with cork or paper and containing fresh water or dilute (0.2 per cent) salt solution. With a small sharp scalpel a narrow strip extending from the region of the pronephros to the tip of the tail is then cut off from the back of the embryo (Fig. 2). This strip contains the medullary tube, including the ganglion crest as far as it is developed, the dorsal portion of the myotomes and the unsegmented mesoderm, and also the dorsal fin fold. With some practice it is possible to cut just between the medullary tube and the notochord, leaving the latter intact. One must count upon a number of failures, but

Fig. 2. Embryo of R. palustris immediately after the removal of the spinal cord. × 9½.

fortunately it is possible to see immediately after making the cut just what has been removed, for in successful cases, on examining the wound surface with a lens, the notochord stands out as a distinct rod with the myotomes arranged alongside (Fig. 4). The operation may also be done successfully with a sharp, fine pair of scissors. In some experiments the thin strip of tissue containing the spinal cord was cut off entirely (Fig. 2); in others it was left hanging at its anterior end, but prevented from healing again to the main portion of the embryo (Fig. 4).

The embryos were kept after the operation in ordinary tap water or placed for a day or two in dilute salt solution, which insures a somewhat more rapid and perfect healing of the wound. This usually takes place, however, without difficulty in any case and even in two or three hours the wound is usually closed. As a control to the study of the further development, normal embryos of the same age were kept side by side with those which had been operated upon, and specimens of each were preserved from time to time for the purpose of studying their internal development.

As regards their external form, the embryos develop in the best instances normally except for the defect produced directly by the operation. Their development is, however, considerably retarded, not only in the region of the trunk and tail but also in the head where the nervous system was left intact. The individuals differ from one another considerably, owing, no doubt, to slight differences in the amount of tissue originally cut away. In the most favorable cases (Fig. 3) the tail is almost straight and shows only a lack of the dorsal part of the axis and the dorsal fin, but as a general rule the tail acquires a marked dorsal bend (Fig. 6), especially in sylvatica larvæ. In other cases, when the notochord has been injured, considerably greater deformity arises; this manifests itself in the crumpling and shortening of the tail, or sometimes even in its almost complete atrophy.

FIG. 3. Palustris larva six days after excision of the spinal cord as in Fig. 2. $\times 9\frac{1}{2}$.

One rather remarkable feature, which shows itself constantly, is the presence of a small portion of the dorsal fin at the tip of the tail (Figs. 3 and 6). Microscopic examination shows that a small portion of the medullary tube is also present at that point. The development of these

structures, lying dorsal to the axis of the tail assumes in some instances considerable proportions. When it is considered that everything dorsal to the notochord was removed by the operation, it is clear that this portion of the medullary tube must have regenerated in an anterior direction from the walls of the neurenteric canal.

The series of drawings (Figs. 4-6), made from one embryo at different stages of its development, gives an idea of the development of the individual. The first cut (Fig. 4) shows the embryo just after the operation. In this case the strip containing the spinal cord was left hanging to the head. The second stage (Fig. 5) is one day older. Here the tail shows a distinct dorsal flexure, due probably to the fact that the distal end of the notochord had been cut out, and considerable growth had taken place before the complete regeneration of the notochord occurred. The cut edge of the small dorsal strip has also healed over and is beginning to coil up in a horizontal plane. Six days after the operation the larva appeared as in Fig. 6. The tail is normally expanded, but still shows the marked dorsal flexure. The small strip of tissue resting on the back has coiled itself up; the dorsal fin belonging to it is well developed. The larva is œdematous and the lymph sinuses are much dilated. This condition is not uncommon in such specimens. Examined more carefully under moderate powers of magnification, the arrangement of the muscle plates in the tail is found normal. The individual segments are V-shaped as usual, although the dorsal arm is shortened by the amount removed by the operation. The primary abdominal muscle is also present and extends anteriorly from the myotomes at the base of the tail, spreading out into a thin sheet in the abdominal walls.

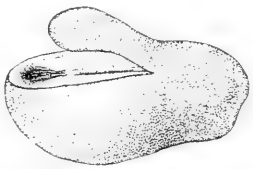

FIG. 4. Sylvatica embryo immediately after cutting the spinal cord. $\times 9\frac{1}{2}$.

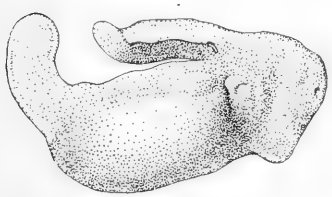

FIG. 5. Same specimen as in Fig. 4, one day after the operation. $\times 9\frac{1}{2}$.

The physiological differences between the embryos experimented upon and the normal ones are marked. While the latter soon acquire the power of movement and respond readily to stimuli, the former remain motionless even when stimulated strongly, except for the movements in several anterior myotomes where the cord had been left in connection with the body. In certain cases the tail exhibited independent twitching movements; in these cases a considerable stretch of the spinal cord was found

to be present. They were, of course, rejected as being open to the suspicion that the nervous influences had not been entirely eliminated. In none of the larvæ without spinal cord was there ever any response to the direct mechanical stimulation of the muscles to be observed with certainty. On the other hand, in the one case (R. palustris) in which electrical stimulation was tried, a marked local contraction of the axial musculature at the root of the tail followed the application of the electrodes at that point. This indicates that the musculature in these instances is capable of functioning. The case is, however, not quite conclusive, because no complete, microscopic examination was made, the specimens having been severely injured during the stimulation.

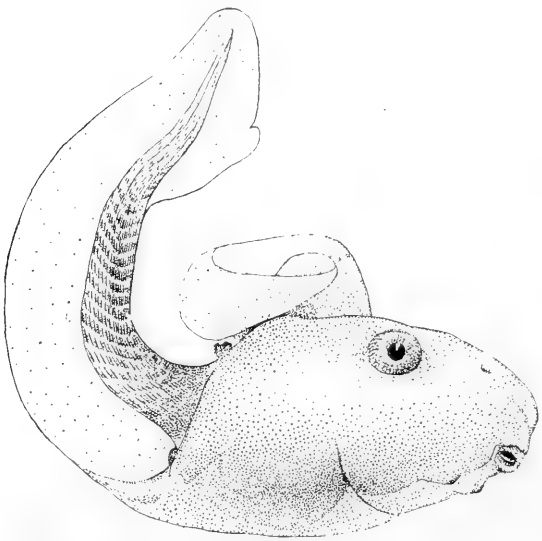

FIG. 6. Same specimen as in Fig. 4. Six days after the operation. $\times 9\frac{1}{2}$.

The examination of specimens in serial sections demonstrates clearly that the effect of the operation is to remove permanently the spinal cord from the greater part of the trunk and tail. Only the small portion at the tip of the tail is present. This part of the medullary cord is, however, in normal as well as in injured specimens, merely an epithelial tube containing no ganglion cells and giving rise to no peripheral nerve fibers. No regeneration of cells takes place from the anterior portion of the cord. The cut end is found to be rounded off and the ventricle entirely closed. The nerve fibers constituting the longitudinal bundles extend, however, in all cases examined, for a considerable distance beyond the limits of the cord. They leave its posterior end and pass in a caudal direction through the mesenchyme occupying the small space bounded by the myotomes, notochord and epidermis. These bundles are thick and very distinct as they emerge from the cord; sometimes they break up into small bundles and in several instances distinct fasciculi could be traced into the lateral branch of the vagus. The bundles gradually become thinner as

they extend further from the medullary cord and finally terminate altogether. Their exact mode of ending could not be determined. Distal to the point of termination of these intrinsic fibers of the cord there are no nerves of any description in the organism, except sometimes the r. lateralis vagi, which, as has been shown, grows out from the vagus ganglion,[4] a structure not affected by the operation. The sense organs of the lateral line are also present in such cases. Spinal ganglia are, as would

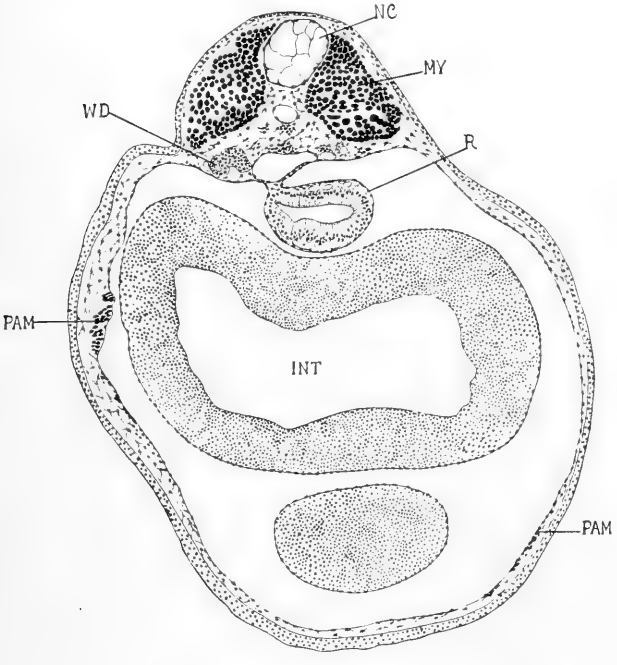

FIG. 7.

FIG. 8.

FIG. 7. Transverse section through the posterior part of the trunk of the specimen shown in Fig. 3; *int*, intestine; *nc*, notochord; *my*, myotome; *p. a. m.*, primary abdominal muscle; *r*, rectum; *wd*, Wolffian duct. ×50.

FIG. 8. Transverse section through the tail of the specimen shown in Fig. 3. *my*, myotome; *nc*, notochord. ×50.

be expected, absent from the entire region distal to the cut end of the medullary cord. In those cases in which the small dorsal strip containing the spinal cord was not cut off, but left attached by its anterior end to the head of the embryo, the longitudinal bundle fibers remain entirely within the walls of the cord and no free nerve fibers whatever are to be found in that part of the embryo where the immediate connection with the cord is severed.

[4] Cf. Harrison, 03a.

The general arrangement of the organs of the trunk and tail is well shown in cross sections (Figs. 7 and 8) of the specimen represented in Fig. 3, which was preserved six days after the operation. The absence of the dorsal fin and the medullary cord is striking. The notochord, which is normally developed, lies almost immediately below the epidermis in the trunk (Fig. 7). The greater part of the axial musculature is intact, only the dorsal portion having been cut off. The arrangement of the fibers in the myotomes is normal, except that the individual fibers are often separated from each other by quite large, clear spaces. In the tail the musculature of the two sides arches over the notochord dorsally (Fig. 8), forming a continuous sheet, horseshoe shaped in section. Examination of sagittal sections of embryos which had lived from five to seven days after the removal of the cord corroborates the results of the observations upon the living specimens, as regards the arrangement of the musculature. The division into myotomes is distinct. The primary abdominal muscle is seen as a band of fibers arising from the ventral edge of the myotomes at the base of the tail, skirting past the bud of the hind leg and spreading out anteriorly into a sheet of cells in the abdominal walls. The anterior portion of this muscle is, as is normally the case in this stage, composed of spindle-shaped cells with little or no contractile substance.

While the above account holds for the best specimens, many cases were observed in which there was much irregularity in the arrangement of the muscle fibres. Such irregularities are more marked in the immediate neighborhood of the scar. They are undoubtedly due to a disarrangement of some of the cells at the time of the operation and to uneven healing of the wound.

The study of the muscular tissue with highly magnifying powers reveals in the best instances a perfectly normal differentiation of its elements. From this condition there are to be found all gradations down to that shown in some of the poorer specimens in which the degeneration of the elements is marked. In the injured embryos there is a distinct retardation of the differentiation of the muscle fibres, corresponding to the slower development of the organism as a whole. In a specimen killed three days after the removal of the cord there is thus but a small amount of contractile substance laid down in the myotomes and the muscle cells still contain a large amount of yolk. Cross striations of the fibrils may, however, be made out. In a series of sagittal sections of an embryo killed six days after the operation the differentiation of the muscle fibers shows a marked advance. The yolk spheres are almost

entirely gone from the myotomes and the fibers are crowded with striated fibrillæ (Fig. 9). The most striking abnormal feature in the individual under consideration is the presence of vacuoles in the axes of the muscle fibers, together with a larger amount of pigment than is usually found. The muscle cells of normal larvæ may, however, show some vacuolization in the axial protoplasm at the time when the absorption of the yolk is about completed. In the injured larva the length of the muscle fibers is not so great as in the normal; many fibers are separated from the neighboring ones by clear spaces. Cross sections of a larva of the same age as the one just described show that the muscle fibers are surrounded by a very delicate membrane, the sarcolemma (Fig. 11). The fibers, which are cut near the end, are filled out entirely by fibrillæ; those cut near the middle show nuclei mostly situated in the axis of the fiber, though sometimes eccentrically placed just beneath the sarcolemma. Vacuoles are also present in the axial sarcoplasm.

The amount of vacuolization shown by the muscle fibers varies considerably in different specimens and even in different regions of the same one. Thus, there are often to be found fibers of perfectly normal appearance, with no vacuoles at all and no other signs of degeneration. Such a fiber, taken from the tail of an individual much like the one shown in Fig. 6, is shown in the accompanying cut (Fig. 10). In the musculature of the limb of the specimen described in the next section there are likewise no vacuoles in the fibers. On the other hand, in some specimens there is not only marked vacuolization, but also alteration of the contractile substance and partial arrest of its development. Blotches

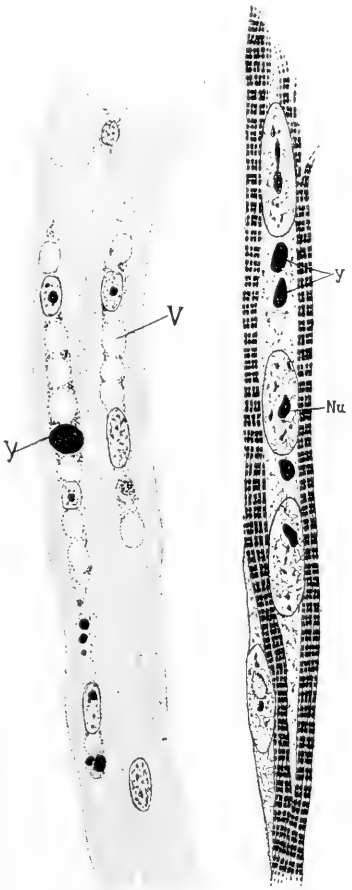

FIG. 9. FIG. 10.

FIG. 9. Two muscle fibers from the root of the tail of a larva from which the spinal cord had been removed six days prior to fixation; *v*, vacuole; *y*, yolk spherule.

FIG. 10. Muscle fiber from tail of larva similar to the one shown in Fig. 6. Killed seven days after cutting the spinal cord. This figure has been reduced to the size of Fig. 9 for comparison, though the magnification is much greater.

of an almost hyaline substance, which stains intensely with Congo red, are found scattered through the musculature in these cases. These conditions are found also in other parts of the specimen where the nervous system is still in connection with the musculature. They cannot therefore be considered as due to the lack of nervous stimuli but rather to unfavorable accidents of the operation.

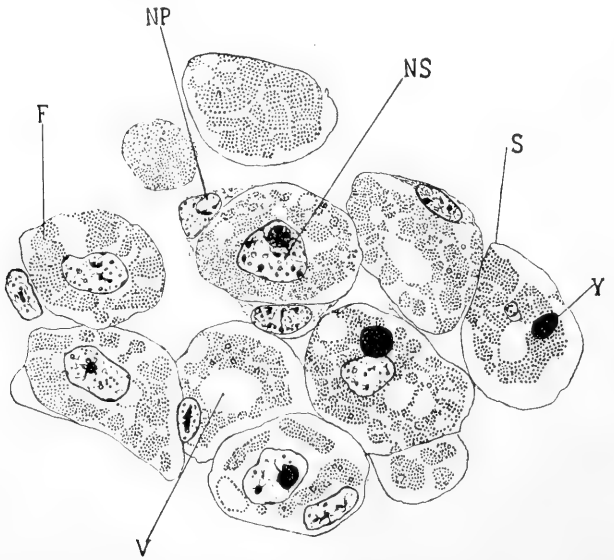

FIG. 11. Muscle fibers in cross section, from the same section as Fig. 7; larva shown in Fig. 3. *f*, fibrillæ; *np*, nucleus of perimysium internum; *ns*, nucleus of muscle; *s*, sarcolemma; *v*, vacuole; *y*, yolk spherule.

The Development of the Hind Limbs without the Presence of Nerves.

While the foregoing experiments suffice to show that the grouping of the muscle fibers into individual muscles takes place without the influence of the nervous system, it might be urged that this is the case only in the muscles of comparatively simple arrangement, such as the myotomes and their immediate derivatives. It seemed desirable, therefore, to test the power of development of the more complicated musculature of the limbs, when the nerve normally supplying it is prevented from growing into it. At my suggestion Mr. H. L. Langnecker undertook to determine this point.

The experiment was carried out as follows: A horizontal slit was made just below the notochord in the axis of the body of a young embryo, in the region from which the hind limb would develop. The wound

was prevented from healing by the insertion of a hedge-hog spine for a few hours, until the cut surfaces healed over. The hole made in this way was found to remain open in the majority of instances, and the spinal nerves were thus prevented from growing out into the limb. Such larvæ live readily for a time, but difficulty was experienced in keeping them alive until the time for metamorphosis. One specimen lived, nevertheless, for this length of time. As regards outward form the hind legs developed fully; all of the segments were normal, as was the number of toes. The limbs had, however, an atrophic appearance and no voluntary movements were ever observed, nor could any response to mechanical or electrical stimulation be obtained. Examination of sections failed to reveal the presence of nerves in the hind limb. Cartilage bone and muscle were normally differentiated. The striated contractile substances filled out the nerve fibers, which were, however, of somewhat smaller calibre than in the fore leg in which the nerves were intact. The individual muscles of the hind limb are clearly defined, but it has not been made out as yet whether all of the muscles normally found in the limb are present in this specimen also. The work will be continued during the present season and a full account published by Mr. Langnecker.

The Development of the Embryo in Solutions of Acetone-chloroform.

For the purpose of drawing or carefully studying living tadpoles it is nearly always necessary to anæsthetize them. Acetone-chloroform has been found to be exceedingly well adapted to this end.[5] It is very easy to manage; a few small crystals added to a watch glass or small dish of water containing the larvæ suffice to stop all voluntary movements, including those of respiration, within a few minutes, while the heartbeat is scarcely affected. The narcosis may be continued as long as desired. On transferring the tadpoles to fresh water recovery takes place quite as rapidly as the narcotization did.

These observations led to the experiment of rearing larvæ under continued narcosis in order to determine the effect of their forced inactivity upon the development of the musculature. It is certain that all functional activity of the muscles is suspended during the action of the drug and also that this is brought about by action upon the nerve centers and not peripherally.

[5] It was at the suggestion of Dr. Abel, who first discovered the anæsthetic properties of acetone-chloroform, that I made use of this drug. Miss Randolph, 00, has shown that it is very useful for the narcotization of many kinds of aquatic organisms. The substance is known commercially as "chloretone."

A few preliminary experiments with older larvæ demonstrated that a 0.02 per cent solution, i. e., two parts of acetone-chloroform in ten thousand parts of water is sufficient to narcotize them completely,[6] and that the action of the heart is not materially altered. Weaker solutions do not completely inhibit muscular reflexes. Solutions of 0.04 per cent and stronger seriously affect the circulation and ultimately cause death.

After these facts had been determined, the experiments bearing upon the problem to be solved were undertaken. Embryos of each species of frog were placed in similar dishes containing water and solutions of the drug of various known strengths, in order that their development in each might be compared. The embryos selected were all in the same stage of development (Fig. 1) and showed no trace of histological differentiation in the nervous system or musculature. In each experiment care was taken to keep the conditions influencing the different sets of embryos as nearly uniform as possible, varying only the strength of the solution. Owing to the volatility of acetone-chloroform, it was found necessary to keep the dishes containing the embryos closed, and to change the fluid every day or two.

Several factors which had a district influence on the success of the experiments manifested themselves during their course. It was found, in the first place, that the embryos of R. virescens suffer least from the action of the drug. Those of R. palustris are somewhat more susceptible, while sylvatica embryos exhibit a considerably more marked tendency towards deformity. Again, it was found that the deleterious effects of the drug were less marked when the temperature was near the optimum for development, for this permitted the embryos to reach the final stages of development in a minimum time.

The results of the experiments may be summed up very briefly. The embryonic development takes place in solutions of the drug, when care is taken to keep the conditions favorable, in an almost normal manner, although distinctly more slowly than in water. The retardation of the development is directly proportional to the strength of the solution. The results in detail may best be presented by giving the original record of a typical experiment.

Experiment 6.

April 20, 1902. Sixteen palustris and seven virescens embryos are put into a 0.03 per cent solution of acetone-chloroform, and eight palustris and three

[6] There is some discrepancy between this result and Miss Randolph's, who makes the minimal dose much stronger. This can perhaps be explained by the rapid volatilization of the substance when kept in open vessels.

virescens embryos into a similar dish containing a solution of the same strength, the latter to be used for testing the irritability. Eight palustris and two virescens embryos are placed under similar conditions in water.

April 22. The temperature has varied between 70° and 80° F. In the palustris embryos kept in water the external gills are sprouting and the blood may be seen circulating in them. These embryos react reflexly to mechanical stimuli; i. e., on being touched by a needle, they first contract the opposite side of the body. The drugged embryos are not quite so far along in their development. The heart-beat is distinct, but there is no circulation as yet in the gills. There is a slight swelling in the pericardial region (Fig. 12). The test embryos react locally to mechanical stimuli (direct muscular stimulation).

April 23. The temperature has been above 80° F. The embryos in the acetone-chloroform solution are developing well. The circulation in the external gills is good. They scarcely give even a local response to stimuli.

April 24. The temperature has been cooler, but above 70° all day. The drugged embryos are developing normally. There is no reaction to stimuli except a faint local one. The solution is diluted to 0.025 per cent.

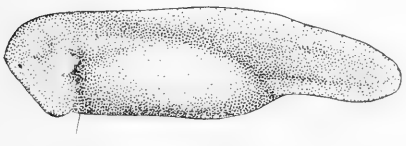

Fig. 12.

Fig. 13.

Fig. 12. Embryo of R. palustris kept two days in a 0.03 per cent solution of acetone-chloroform. ×9. P = pericardium.

Fig. 13. Control embryo kept two days in water. ×9.

April 25. Temperature this morning, 68°. The circulation is well established in the tails of the palustris embryos. The virescens embroys are not quite so far advanced in their development. There is no reaction to stimuli, except a very feeble local one.

April 26. Solution of drug diluted to 0.02 per cent. The circulation in the tail of the virescens embryos is well established. There is only very slight local reaction to stimuli.

April 27. The control specimens kept in water are now feeding and passing fæces. The drugged embryos are slightly swollen. The coils of their intestines are not wholly normal. The heart action is good. Three palustris and one virescens larvæ are preserved. The others are put into fresh water for recovery.

4.38 P. M. Larvæ put into fresh water.

4.42. No reaction to stimuli.

4.49. Virescens larvæ react with a quiver or jerk. Palustris larvæ do not react at all.

4.55. Virescens larvæ are able to swim across the dish. Jaws are moving. Gasping respiratory movements. Palustris larvæ do not react.

5.15. Palustris larvæ do not react.

5.29. Some of the palustris larvæ react with a jerk or two.

545. All of the palustris larvæ react to stimuli. Several able to swim across the dish.

FIG. 14. Embryo of R. palustris kept five days in a 0.03 per cent solution of acetone-chloroform. ×9.

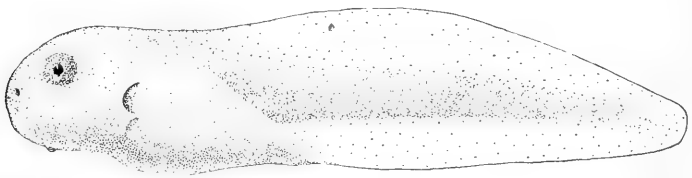

FIG. 15. Control embryo kept five days in water. ×9.

May 6. The recovered tadpoles have been kept in a large aquarium, with plenty of food. The palustris larvæ appear normally formed when seen from above and from the side. One has remained very small. The intestinal coils are not normal. Of the virescens larvæ, two are normal looking, except for the intestinal coils. One is very much swollen on the sides, due probably to distention of the lymph sinuses.

The histogenesis of the muscular tissue was followed in a series of specimens, taken from the above and other experiments and preserved from day to day. The embryos were fixed in mercuric chloride and acetic acid and the sections stained for the most part in Heidenhain's iron hæmatoxylin.

Experiment 6. Palustris embryo two days in 0.03 per cent solution.— The muscle cells in the myotomes are normal. They still contain a large amount of yolk but no vacuoles. Contractile fibrillæ are present in considerable quantity.

Experiment 6. Palustris embryo three days in 0.03 per cent solution.— Corresponding with the general retardation of development as compared with that of the control embryos reared in water, the development of the individual muscle cells is retarded (cf. Fig. 16 and Fig. 17). There is more yolk in the muscle fibers of the drugged specimen; the striations of the muscle fibrillæ are in corresponding myotomes less distinctly marked. The individual fibers are not so slender as in the normal control. When a comparison is made between the less differentiated myotomes in the tail and those in the trunk of the normal specimen, it is seen that the difference in the clearness of the striations is merely an evidence of the difference in the degree of development. There is a slight vacuolization of some of the muscle fibers in both

of the embryos. The basis of comparison of the two specimens is rendered all the more exact by the fact that the sections of both were run through the staining fluids simultaneously.

Experiment 6. Palustris embryo, five days.—There is a marked increase in the vacuoles in the axial sarcoplasm of the muscle fibers, while in the control specimen, reared in water, there is but very slight vacuolization.

Experiment 6. Palustris embryo, seven days.—In this specimen the yolk is practically gone. The vacuolization of the fibers of the myotomes is marked. The fibers are separated from each other by clear spaces. The jaw muscles show some vacuolization, but in a much less marked degree than in the myotomes. The striated fibrillar substance is also well marked in the former.

Experiment 7. Larvae of six days.—Two specimens, one of which had been kept in a 0.025 per cent solution, and one control reared in water were imbedded side by side and cut and mounted together. The contrast in the muscular tissue in the two specimens is not marked. There is more vacuolization in the drugged larva, Fig. 18, but this characteristic is much less marked than in the specimen just described. This condition is due in all probability to the circumstance that the solution of the acetone-chloroform was slightly weaker than in the former case, and also to the fact that, owing to the high temperature, the larva had attained its development in six days of exposure to the drug, instead of in seven, as in the former instance.

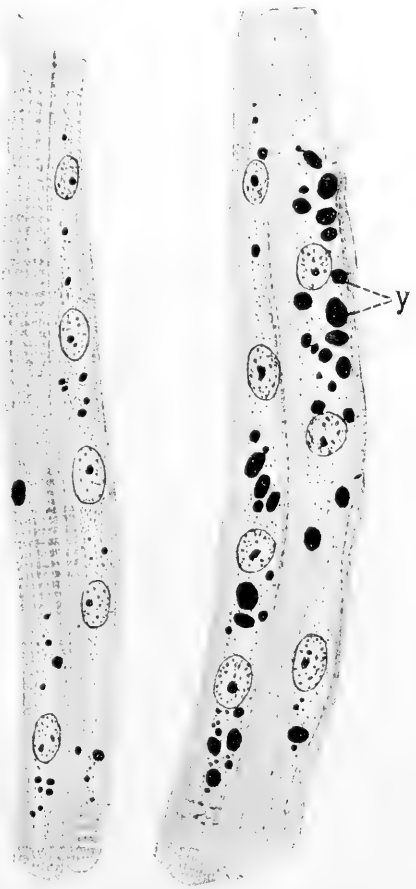

FIG. 16. FIG. 17.

FIG. 16. Two muscle fibers from the ninth myotome of a normal palustris embryo, three days older than the stage used in the experiment.

FIG. 17. Two muscle fibers from the myotome of a palustris embryo kept for three days in a 0.03 per cent solution of acetone-chloroform. *y*, yolk spherule.

The most striking feature of the experiment described in full above is the extraordinary rapidity of the recovery from the action of the acetone-chloroform; in several other similar experiments the recovery was even more rapid. Thus, in one instance (experiment 1), a virescens embryo,

reared in 0.02 per cent solution, had in five minutes recovered sufficiently to swim several strokes, and in seventeen minutes the co-ordinated movements of this specimen could not be distinguished from those of a perfectly normal larva. In other cases the recovery of palustris larvæ was found to be considerably more rapid than in the experiment just described in full though it was never so rapid as is the case with virescens larvæ. It is clear then that the mechanism requisite for carrying out the complex muscular movements of locomotion and respiration develops normally without ever having functioned, although in the normal development of the embryo, the acquirement of this power is a gradual one, being accompanied by the frequent activity of the parts.

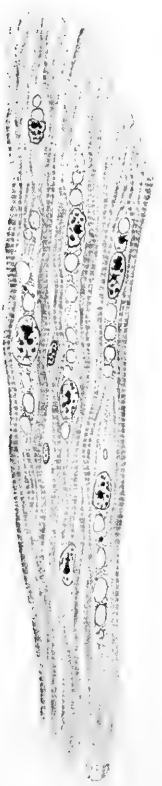

FIG. 18. Muscle fibers from myotome at base of tail of larva kept six days in acetone - chloroform.

The irritability of the developing embryos was tested in the experiments from day to day by stimulation with a needle-point. No reflex response was ever observed at any stage in embryos reared in solutions of 0.02 per cent, except in a few doubtful instances. Nevertheless it seemed safer to experiment with somewhat stronger solutions (0.025 to 0.03 per cent), in which no embryos ever manifested any reflex activity whatever. Electrical stimuli were not tried, but it was observed that the drugged organisms were not sensitive to chemical stimuli, for in putting them into the ordinary fixing fluids, such as mercuric chloride or formalin, no movements were ever observed, while normal embryos contract their muscles violently. The irritability of the muscle itself remains, however, even in embryos kept in the stronger solutions of the drug, but the effect of direct stimulation of the muscles may readily be distinguished from that of the indirect or reflex irritation. The former is evidenced by a sharp tonic contraction of the myotomes on the same side of the body and immediately at the point of application of the needle prick; moreover, this type of contraction takes place only on strong stimulation, often only when the muscle is actually pierced by the point of the needle. The reflex response of normal embryos is quite different from this. If one stimulates a young embryo by lightly touching it on one side of the body, the first response is a general contraction of the myotomes usually on the opposite side of the body, followed by the alternate contraction of the two sides, which results in a co-ordinated

swimming movement. These experiments afford therefore a corroboration of the conclusion drawn from experiments on higher animals that the acetone-chloroform acts upon the nerve centers and not peripherally.

The general retardation of the development of the drugged organisms results probably in the first instance from disturbances in the metabolism of the cells, possibly in their diminished power of oxidation. It is at least justifiable to assume this in view of our knowledge of the action of related substances, such as chloroform, upon adult organisms. While the external gills of the embryo are functional, this is perhaps the sole cause for the slower development. Later, when the internal gills are developed, the larvæ, which are normally dependent on the respiratory movements for the proper aeration of the blood, must lack an adequate supply of oxygen. This contributes to further delay in development and no doubt is the cause of some of the deviations from the normal course, which manifest themselves more clearly in this late period.

The differences in external form between the normal and the drugged embryos are not great. There is often a considerable effusion of fluid into the pericardial cavity at an early period, causing an unusual swelling in this region (cf. Fig. 12 and Fig. 13); and besides, the bodies of the drugged larvæ are usually somewhat swollen. The caudal fin of these specimens fails to expand as fully as in normal ones (cf. Fig. 14 and Fig. 15). The œdema and the pericardial effusion are no doubt due to weakened heart action, and this may affect also the circulation in the tail to some extent, resulting in a slight arrest of development. The vacuoles, which form in the axial sarcoplasm of the muscle fibers, may also be accounted for by disturbances in the circulation.

Discussion of the Results.

In the first series of experiments described above, the spinal cord of the embryo was removed before the histological differentiation in either the muscular or the nervous tissue had begun. From the very beginning of the visible changes in structure, which transform a simple mesodermal cell into a muscle fiber, the isolation of the musculature from the nervous system was complete. All chance for the exertion of any peculiar formative stimulus emanating from the nervous system as such was eliminated; and likewise, owing to the consequent paralysis of the muscles in question, any possible stimulus resulting from the functional activity of the muscle itself was excluded. Still the differentiation of the contractile substance took place in normal manner, as did the grouping of the fibers into individual muscles. Just as Schaper's experiments have shown that the brain as a nerve center exerts no general formative

influence upon the development of the organism as a whole, the present experiments demonstrate that the nerve elements normally innervating a muscle play no part in its morphogenesis.

This experimental demonstration of the independence of the developing muscular tissue may be regarded as crucial evidence against the general correctness of the view held by Neumann, 01 and 03, that the first development of the muscles takes place under the influence of the nervous system through the agency of the motor nerves. Herbst's, 01, assumption of a formative stimulus proceeding through the sensory nerves is also shown to be erroneous. Of course there is the possibility to be considered that the conditions obtaining in mammals differ, in regard to the action of the nervous system, from those in the frog; but this is not likely, and in view of the relative activity of the developing tadpole, as compared with the mammalian fœtus, any differences between the two would most likely be in favor of a more important influence being exerted by the nervous system in the former than in the latter.

The second series of experiments is like the first in that the effect of possible functional stimuli is entirely eliminated, although the possibility still remains that special formative or trophic stimuli, if such exist, are not interrupted by the action of the acetone-chloroform. While, therefore, the latter experiments are in themselves not so conclusive as the former in proving that the histological differentiation of the musculature is independent of the action of the nervous system, the similar results in the two series would indicate that the two methods of elmination of nervous action, the operative and the chemical, are as a matter of fact equivalent. The experiments with acetone-chloroform have the additional value that the function of the nervous system may be restored by the removal of the drug from the organism. In this way the functional power of the complex nervous and muscular mechanisms, which carry out the movements of swimming and respiration, may be tested. The surprisingly quick recovery—or better, since the musculature had never shown any activity—the quick acquisition of the power to carry out these movements, shows that the mechanisms in question develop in perfect order, without the influence of normal function in each successive stage. The organism in which this takes place is one which is normally very active, and one in which the power of locomotion is only gradually acquired. While the above fact cannot but strike one as remarkable, it is, nevertheless, on the other hand, in accordance with what should in reality be expected, for such complex mechanisms as, for example that used in respiration, develop in the mammallian embryo during intrauterine life without ever having been brought into action.

While it has been emphasized in the foregoing that the building up of the musculature takes place normally even in the absence of connection with the nervous system, it is not to be lost sight of, that in all of the experiments certain signs of interference with normal development and of degeneration make themselves apparent. The general retardation of the development of embryos reared in acetone-chloroform may, however, be accounted for, as pointed out above, by the direct action of the drug upon metabolism and upon the heart. The most noticeable degenerative change in the embryonic muscle, the appearance of vacuoles in the axial sarcoplasm, may also be explained as due to disturbances in the circulation. That the vacuolization of the embryonic fibers is not due specifically to the removal of nervous influence is shown clearly by the fact, to which Dr. Knower has called my attention, that an exactly similar condition supervenes in the musculature of frog embryos from which the heart had been removed at an early stage. Much of the interference with the normal processes of development may therefore be set down as due to influences other than the changed relations with the nervous system, though it is not impossible that the disturbances are due to some extent to the latter cause. This would not be remarkable, however, in view of the well-known fact that in the adult a muscle undergoes atrophy after its nerve supply is cut off.[7]

We must, in fact, consider the embryo not merely as a developing organism, in which the parts are important potentially, but also as an organism, which in each stage of development has functions to perform that are of importance for that particular stage. If these functions are interrupted, as they are in the present experiments, we can but expect to find, that side by side with the constructive processes which build up a muscle fiber out of an undifferentiated muscle cell, and which, as the experiments show, take place quite independently of the nervous system or the stimulus of function, there also take place certain degenerative changes due to the absence of these influences. The results of experi-

[7] The morphological changes which take place in a muscle after neurotomy have been the subject of numerous investigations, of which a full review has been given by Stier, 97. The most pronounced changes are diminution in the caliber of the fibers and proliferation of the sarcolemma nuclei. Ricker and Ellenbeck, 99, find also vacuolization of the fibers and other signs of œdema. Ricker, 01, explains the changes which take place as due primarily to the interference with the normal working of the vasomotor apparatus of the muscle. De Buck and de Moor, 03, who have studied the subject most recently, emphasize the regressive changes of the muscle fiber itself, i. e., its return to the embryonic condition, and consider the changes to be due to the lack of functional stimuli.

ments on the sense organs of the lateral line, after destruction of their nerve supply,[8] bear out the correctness of this view.

Long ago Roux, 81 and 85, suggested that the development of the organism could be divided into two periods, the one an organ-forming period and the other one of functional development. In the first the organs are formed and brought to that condition in which they are capable of beginning a specific function; in the second period, in which the organs exercise their specific function, their further perfection is helped by this activity and interfered with by its absence. While the general aptness of this distinction is apparent, the present study shows that there is not only an overlapping of the two stages in different systems of organs, as Roux pointed out, but also in the same organs; even in one and the same muscle fiber, as shown above, the tendencies of the two periods may manifest themselves side by side. It must nevertheless again be emphasized that all of the constructive processes involved in the production of the specific structure and arrangement of the muscle fibers take place independently of stimuli from the nervous system and of the functional activity of the muscles themselves.

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A BONY SUPRACONDYLOID FORAMEN IN MAN.

WITH REMARKS ABOUT SUPRACONDYLOID AND OTHER PROCESSES FROM THE LOWER END OF THE HUMERUS.

BY

THOMAS DWIGHT, M. D., LL. D.,

Parkman Professor of Anatomy at the Harvard Medical School.

WITH 1 PLATE.

This foramen (Fig. 1) which, so far as I know, is unique in literature, occurs in the left humerus from the body of a white woman aged 57. There is neither supracondyloid foramen nor process on the right one. Otherwise the humeri are very symmetrical, and present no signs of pathological ossifications. The length of each is 28.5 cm. The angle of torsion of the right humerus is 157 degrees, and on the left 160 degrees. The process inclosing the foramen springs from the inner surface about midway between the internal and anterior borders 62 mm. above the lowest part of the trochlea. It arises by an extremely thin triangular expansion, about 1 cm. broad from above downwards, and is continued as a slightly convex arch 32 mm. long, measured on the convexity, to end in another triangular expansion some 4 mm. broad on the anterior surface of the internal condyle, 2 cm. above its lower border. The foramen, therefore, is bounded wholly by bone. The process is an extremely delicate structure, especially in the upper two thirds, where its thickness is that of paper. The lower end is from 1 to 2 mm. thick. The process is twisted in its course, the expansion at its origin facing outward and forward, and that at its end forward and inward. The thinner part above passes into the thicker part below without any change of character.

The median nerve ran through the foramen. The brachial artery passed over the origin of the process. The brachial artery and its branches were very small; the anterior circumflex was very minute, possibly represented by two twigs, the posterior circumflex was represented by a branch from the superior profunda. At about the usual place of division the brachial gave off a radial artery of about half the diameter of the ulnar, which latter seemed to be the direct continuation. The

median nerve arose in the usual manner, but at about the middle of the arm passed *behind* the brachial artery from without inwards, and at the lower third became separated from it. It passed through the foramen, lay between the pronator radii teres, and the brachialis anticus, and entered the forearm between the two heads of the pronator. The origin of this muscle was continued into the lower third or more of this process while the brachialis anticus received fibres from about a corresponding area at the upper end, the middle of the arch having no muscular fibres and appearing as a white line.

There can be no question that this bony arch represents the process with its fibrous continuation which bounds the occasional opening in man representing the widely distributed supracondyloid foramen of animals. (I may mention in passing that the fibrous band is not constant. My experience in this respect agrees with that of Nicholas (1)). In the first place the process that bounds the foramen occurs at the normal point of origin of the supracondyloid process. This, according to Otto (2), is in a line from the inner border of the trochlea to the anterior border of the greater tuberosity. Testut (3) accepts this and adds another line of his own from the groove in the trochlea (*gorge de la trochlée*) to the middle of the articular surface of the head. In point of fact the course of these lines must be far from constant; but if both these statements be correct, as they approximately are, the process must be at the point of intersection of these lines. Although this is true, the process is lower than usual. I have said it arises 62 mm. above the lowest point of the inner border of the trochlea, the measurement being taken from the highest point of the origin of the process. Testut gives the average distance of eight cases as 71 mm. and Nicholas that of six as 73 mm. Moreover, the latter, at least, placed his starting point at the middle of the base of the process. Ruge (4) declares that according to all experience the position is a constant one; which is practically in accord with my own less extensive observations. There are, however, certain exceptions to be mentioned later. Another important fact is that the median nerve passes through the foramen, *i. e.,* under the process. It may be asked whether this is a real foramen; that is to say: was the strip of bone bridging it over either laid down in cartilage or formed by the early ossification of the completing band, in contradistinction to a quasi-accidental ossification of that band in adult life? In other words, have we at last found the supracondyloid foramen in man? I incline very strongly to consider it a real foramen, and probably one formed by a cartilage. The process usually, when it is more than a ridge or a tubercle, is thick at its base and narrows to the point which

may or may not expand into a knob. It is much stronger than the ligament continuing it. Here, on the contrary, the border of the foramen is thicker below than above. So far as I can remember, all the connecting bands which I have seen, or of which I have seen figures, run straight or even in a concave line to the inner condyle; this, on the contrary, is convex. It is worth noting that in the few supracondyloid processes which have been observed in children ossification begins very early. Thus Macalister (5) mentions a specimen in the Cambridge Museum from a child 27 months old, in which the process is 3 mm. long, and both it and the faint ridge above are ossified. Cunningham (6) has seen the process in both arms of a child of three: on one side 4 mm. long, on the other 3 mm., and both completely ossified. More remarkable still, he has seen it in both arms of a full-time still-born child. "In both bones the process is 5 mm.; and further, it is fully ossified from base to tip. From this it would appear that the supracondyloid process is ossified along with the diaphysis, and from the same center; and further, that its ossification is completed at an extremely early date." There is, I think, every reason to believe that this arch was originally cartilaginous. The case most nearly approaching this, which I am acquainted with, is that reported by Tandler (7). It was also found on the left arm of a woman. The arch, which was bony in the middle and fibrous at both ends, passed over both the artery and the nerve. This implies an early cartilaginous arch incomplete at the ends.

A very thorough examination of the literature has failed to reveal the record of any similar case in man. It is perhaps less surprising that it has now been observed than that it has not been observed sooner.[1]

A rather curious paper by Solger (8) has raised the question whether all processes which at first sight seem to be supracondyloid have the same significance. He describes a process which he calls *anterior sive medius* about 1 cm. long, hooklike, and directed inwards, arising about 4 cm. above the capitellum (capitulum), from which a dense cord of fat,

[1] It is hardly conceivable that any anatomist who should have met with such a specimen should not have made it public. I am told by a competent anatomist that he saw a foramen several years ago in a laboratory in Vienna. It was also reported to me on the authority of a student that there is a similar specimen among the Indian bones in the Peabody Museum at Cambridge. With the kind help of Dr. Farabee of the Museum I searched for it in vain for some three hours. Dr. Farabee thought that we examined nearly a thousand humeri. I should hardly dare to place the number so high; but it is worth noting that among several hundred Indian bones we found only two instances of a supracondyloid process, one of which was small, and the other smaller. I imagine that the foramen seen by the student was above the trochlea.

which he considers an accessory head of the muscle, ran into the brachialis. The pronator teres, the vessels and the median nerve showed no peculiarity. According to him, "Such abnormal processes with abnormal prolongations of the muscles attached to them may spring, as is well known, from various parts of the diaphysis above the inner condyle." Solger considers this and two or three others, which he thinks he has found in literature, as intermediate between the internal supracondyloid process and the rare external one. The cases he refers to are two out of five reported by Gruber (9), and one out of four reported by Turner (10). Having consulted the original papers, I am far from convinced. Gruber himself says that his five new cases presented the same or similar features as the preceding forty-two. Turner states distinctly that in all four cases the process arose from the inner part of the shaft, and that in all the median nerve passed under it. Perhaps the most important peculiarity of one of his cases, presumably the one referred to, is that no fibres of the pronator came from the process nor from the band. The situation of the process in Solger's own case is certainly very remarkable, but I cannot see that the cases he cites belong with it. On the other hand, Bertaux (11) in the same year reported three cases which seem to support Solger's views. Unfortunately there is no account of the soft parts. Two of the specimens are from the same skeleton. The right process is flattened and triangular, with a long base, continuous above with the anterior border of the bone,[2] and prolonged below to the inner border of the coronoid fossa. The upper and lower borders of the process are so symmetrical that it points neither upward nor downward, but is turned inward so as to form something of a gutter. The left one is similarly placed but is larger and with less symmetrical borders, the upper one being more nearly horizontal and also rougher. The third instance is unilateral, on the left arm of a man of 27. The process has the usual appearance, extending hook-like downwards and inwards, the only important peculiarity being that it seems to be continuous with the anterior border. Peculiar processes, not easy to interpret, certainly arise in this region.

The following instance is, perhaps, worth reporting, though unfortunately I have no data beyond those offered by the macerated humerus recently added to the Warren Museum. The specimen (Fig. 2) came from a white man aged 50, who evidently was of very powerful frame. The humerus is strong, and the muscular ridges well developed. The

[2] "Elle semble s'insérer sur un dedoublement du bord antérieur, soulevé fortement a ce niveau."

anterior border of the humerus, instead of subsiding as it approaches the lower end, becomes more and more prominent and is continued into a stout process slanting downwards, forwards and somewhat inwards to end free above the inner half of the trochlea. The vertical distance from the under side of the root of the process to the level of the lowest point on the inner border of the trochlea is 4 cm. The lower border of the process measures 16 mm. It is more difficult to measure the upper border, as it has no definite beginning. It may be said to be about 25 mm. The process is compressed from side to side, the vertical diameter being about 11 mm. and the transverse about 6. It is somewhat enlarged at the free end, which is rough and irregular, and rather suggestive of having been covered with non-articular cartilage; the bone is otherwise healthy, but the shape at the lower end is modified by the exaggeration of the anterior border, which is, as it were, pulled forward by this process. The posterior surface of the bone shows the effect of the distortion, being hollowed above the olecranon fossa to a remarkable degree. This very certainly is a congenital malformation, and no post-partum pathological exostosis. If it made a foramen at all it must have been by some connection with the ulna, but the appearance of the joint does not indicate any limitation of motion. It is hardly conceivable that it formed any connection with the internal condyle. Its inner aspect is slightly grooved as if it may have rested against the artery and nerve. It certainly is not an internal supracondyloid process. It might be called an anterior or middle one, were the term considered justifiable. Gruber would have called it a false internal supracondyloid process. I cannot help thinking that Poirier (12) must have met with some such process as this when he speaks of having witnessed the removal of a supracondyloid process that interfered with the motion of the joint. It is not credible that the ordinary supracondyloid process should do this.[3] The same may be said of processes which are easily felt during life. I examined the body on which this foramen was found before dissection with a special view to supracondyloid processes without detecting anything uncommon.

I hesitate to agree with Solger in considering this as intermediate between the internal supracondyloid processes and the "much rarer external ones." I do not admit a *middle supracondyloid process.* Ber-

[3] "J'ai pu sentir l'apophyse sur le cadavre entier et j'ai vu, à Londres dans le service de Lister, enlever une apophyse très developée qui, faisait saillie sous la peau et génait les movements du coude: il fallut détacher les faisceaux du rond pronateur qui s'insćraient sur le crochet osseux."

taux's observations prove, however, that the position of the usual process is not fixed. Is it possible that it may wander so far as to appear over the external condyle?

The *external supracondyloid process* rests, so far as I know, on the solitary observation of Barkow (13). It is not surprising that though the reference to Barkow's paper is common enough, few seem to have any definite idea of what he described, as his observations are not easily accessible. Having had the advantage of seeing the original, which is in the Surgeon-General's Library at Washington, I give a photograph (Fig. 3) of his figure so that others may judge for themselves of this process. Gruber (14) is very severe in his criticisms of Barkow. The process, he says, is neither in the place it should occupy were it the analogue of the process in mammals it is held to represent; it is in no relation to the radial (musculo-spiral) nerve; it points downwards instead of upwards. I am not quite convinced that it is impossible that the nerve should pass under this process, though it certainly is placed below the usual course of the nerve. It is probable that it is a mere irregular ossification of the external supracondyloid fibrous tissue; but after all it has a decided resemblance to the internal supracondyloid process. I believe that it is of no significance.

In conclusion I would say something as to the explanation of the occasional appearance of the supracondyloid process or foramen in man, much discussed as this question has been. I had the honor of reading a paper on the "Significance of Anomalies" before the Association of American Anatomists in 1894 (15). One of the instances I chose was the supracondyloid process. While I could offer no satisfactory explanation, it seems to me that I showed well-nigh insuperable objections to the common plan of calling them reversions. I then said, "It is clear that if an anomaly in man is to be called a reversion, either the species in which it is normal must have been in the direct line of ancestry, or there must have been a common progenitor." I am inclined now to add that it is reasonable to expect that this common progenitor should be, as one may say, somewhere within call. I also laid stress on the argument that similarity of structure does not necessarily imply common descent; and this is true when we consider the normal structure of animals of different orders, or even I may say of different classes, as well as the variations. Very valuable work has been done by distinguished colleagues since then. Professor Huntington (16) has emphasized the occurrence of such phenomena and has stated the matter with great clearness. Treating of muscular variations he distinguishes three kinds. *Archeal* reversional variations repeat conditions which are not found in

the mammalia, but which appear homologous with structures in other vertebrates and indicate a reversion to the common vertebrate type antecedent to class distinctions. Less far-reaching are *progonal* reversional variations in which the observed structure is normal in no species of that order, and consequently points to the common class stem. *Ataval* reversional variations represent structures which though not normal in the species in question exist in species of the same order. The supracondyloid process, according to him, belongs to the first of these classes; but the question at issue is, whether this method affords any solution of the difficulty. Now the possibility of a reversion is not in the slightest established by calling it archeal; on the contrary it may be said that by defining the dimensions of the gulf to be passed the probabilities of a leap over it become less conceivable. In short, according to the general teaching, it seems to be claimed that putting aside alleged progressive variations and such as for want of a better word may be called accidental, there is no principle to account for variations save reversion. But the difficulty is not yet fully stated; for the problem is not to account for the supracondyloid process only, but for all the variations of bone, muscle, viscus, etc., that occur in man or in any animal. So far as they have been studied we do not find any universal concurrence in the evidence; and yet it is essential to the theory that there should be no contradictions. I have from the first been much impressed by the passage in the late Lord Salisbury's (17) Oxford address concerning Mendeléef's law according to which "elements can be divided into families of about seven, speaking very roughly: that those families all resemble each other in this, that as to weight, volume, heat, and laws of combination the members of each family are ranked among themselves in obedience to the same rule. Each family differs from the others, but each internally is constructed upon the same plan." What was a weakness in this theory "was turned into strength," to quote again his words, by the discovery of certain elements which were wanting in some of the groups when the law was first announced. He continues: "If these were organic beings all our difficulties would be solved by muttering the comfortable word 'evolution'—one of those indefinite words from time to time vouchsafed to humanity, which have the gift of alleviating so many perplexities and masking so many gaps in our knowledge." Physics not being in my line, I thought it advisable to inquire of an authority whether this were correct, and was assured in reply that Mendeléef's law had been confirmed and strengthened since Lord Salisbury's address and is now used as the working hypothesis. If, then, we have such curious resemblances in non-organic nature, why should the mere fact of life put aside the possi-

bility, or rather the probability, of an analogous state of affairs in animals? We find similarity of plan where inheritance is excluded, ergo inheritance is not the sole cause of similarity, whether we deal with the normal condition or with variations. The old idea of type, abused and made ridiculous as it has been, is not all error.

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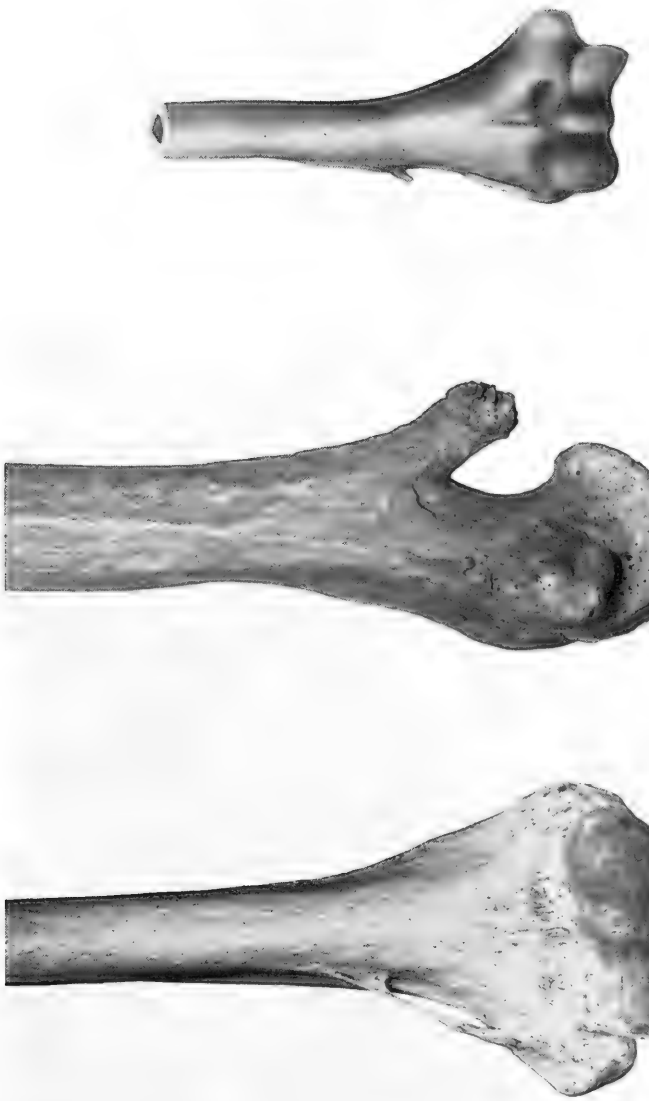
EXPLANATION OF FIGURES ON PLATE I.

Fig. 1.—Supracondyloid foramen seen not directly from the front, but a little from the outside.

Fig. 2.—Peculiar process from anterior border. Inner aspect.

Fig. 3.—Barkow's "external supracondyloid process."

THOS. DWIGHT.



ON THE DEVELOPMENT AND NATURE OF THE NEUROGLIA.

BY

IRVING HARDESTY.

From the Hearst Anatomical Laboratory of the University of California.

WITH 5 PLATES.

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Weigert's paper of 1895 and the investigations stimulated by it have led to the conclusion that the neuroglia as found in the adult nervous system presents two general forms:

First, the more plastic protoplasmic form. This occurs either as masses of more or less modified protoplasm enclosing one or several nuclei and having a more or less definite shape—"neuroglia cells"—or it occurs in the more accumulated and somewhat different form of the substantia gelatinosa.

Second, it occurs in the form of the neuroglia fibers, which are in no sense cell processes, but rather are both morphologically and chemically different from the protoplasm. However, they are derived from the protoplasm, though the manner of their origin is not well understood.

During a study of the neuroglia as found in the nervous system of the elephant (Hardesty, 02), some appearances were noted which seemed suggestive of the earlier form of the tissue and the processes by which the neuroglia fibers are developed. The chief purpose of that paper was to describe the adult form of the tissue as found in the spinal cord of the elephant and to compare it with the more familiar appearances in the adult human nervous system. In addition to this, however, attention was called to evidences indicating (1) that the neuroglia tissue can in no sense be looked upon as composed of independent, or even individual,

"neuroglia cells," but consists rather of an early formed protoplasmic continuum, or syncytium, extending throughout the confines of the central nervous system and in which the nuclei are situated at irregular intervals and in irregular numbers; (2) that the later formed appearances of the tissue, usually described as "neuroglia cells," are simply masses of this syncytium more or less isolated by being molded into the interspaces resulting from the ingrowth and enlargement of the nervous elements, the "processes of the cells" being only the more attenuated portions of the syncytium connecting contiguous larger masses occupying larger interspaces; (3) that finally the neuroglia fibers, or that form of the mature neuroglia which is differentiated by the special neuroglia stains, result from a transformation of the syncytial substance. The fibers in the adult are of irregular and indefinite length. A single fiber may frequently be traced through the domain of several "neuroglia cells."

These impressions were obtained chiefly from favorable preparations of the adult tissue. To observe the processes by which the neuroglia arises and its fibers develop, the study must of necessity deal with different stages of the growth and development of the organ containing it. This paper is an attempt to describe certain of these processes thought to be indicated by conditions found in the developing material.

In general, the literature touching the subject is unsatisfactory, from the fact that in most cases the authors deal with other than the special features herein concerned. Often an author's statements, and his illustrations especially, show that he has seen certain of the features I shall try to describe, but usually giving special attention to the developing nervous structures, he either leaves the supporting tissue unnoticed or describes its appearance from a different point of view. In fact, the idea of a syncytium in these phases of development seems to be comparatively new in the literature.

In order to avoid possible confusion in its use, it is perhaps best to define how the term syncytium is used in this paper: Wherever there occurs a division of the nuclei without a corresponding division of the cytoplasm there results a syncytium, or a condition in which the nuclei are distributed in a common mass of cytoplasm. The nuclei may or may not exhibit a regular form of arrangement; the mass itself may or may not have definite shape. This definition may be strained to include the giant marrow cell or the striated muscle fiber. In these there is a more or less definite shape with a more or less definite arrangement of the nuclei. The periblast of certain early embryos has neither. Mall, 02, especially describes the early form of the connective tissues of the body as that of a syncytium which results from the fusion of the mesen-

chymal cells. Here is a syncytium with its nuclei variously distributed and with a shape and boundaries none other than those of the entire body of the animal itself.

As to the processes by which the syncytium may be formed, His, 98, in his paper "*Ueber Zellen und Syncytienbildung,*" states that a syncytium may occur either in consequence of delayed formation of the cell membranes or it may, secondarily, arise from a fusion of cells already formed. In the first case the syncytium may or may not disappear through a later formation of cell boundaries. Mall's description of the connective-tissue syncytium deals with embryos in relatively late stages and, in this case, it is needless to state that it maintains. It is a stage in the development of a tissue, the very nature of which forbids its breaking up into individual cells. Portions of the substance of the syncytium may become converted or differentiated into structures differently arranged and chemically different from the remaining portion. Fibrillæ are developed in the muscle cell, and Mall has described the development of different forms of connective tissue (fibrous, etc.) from the syncytial protoplasm.

Material and Methods.

Pig material has been used almost exclusively because the different stages required could be more easily obtained. The observations have been confined to conditions found in the spinal cord alone and in all cases the pieces were taken from the cervical region. Preparations of the adult pig were supplemented with similar preparations of human spinal cord and with that of the ox. In order to follow the developmental changes as closely as possible, preparations were made from quite a number of pig embryos and fœtuses. The smallest I was able to obtain measured 5 millimeters, unflexed. Beginning with this, the series involved about twenty different stages, the first seven being taken at much closer intervals than the remainder. The series terminated with a fœtus of 28 centimeters, suckling pigs of two weeks, and specimens from two adult hogs. Up to 10 millimeters the measurements could be taken from head to tail; after that "crown rump" measurements were taken in the usual manner.

During, and for a time after, the flexion of the embryo, measurement is a very unsatisfactory method of expressing age in pigs. A flexed embryo may measure only 7 millimeters, when an unflexed, and evidently younger one, may measure 8 millimeters. Also from 15 to 30 millimeters, pigs giving the same crown rump measurement may

vary greatly in bulk, and, evidently, in age. Whenever possible, attention was paid to the thickness as well as the length of the specimens.

Only one method was employed with the first three specimens of the series. They were fixed in Carnoy's (Van Gehuchten's) fluid, and thin paraffin sections were stained with hæmatoxylin and counterstained with Congo red, the latter being an excellent cytoplasmic stain and having been previously found very efficient to bring out cell boundaries when such are present. Pieces of the spinal cord of the remaining of the series were prepared by the special neuroglia method of Benda, **00,** the procedure followed being that employed by Huber, **01.** In addition, however, at various intervals in the series, pieces were prepared as were those of the first three stages. Also Mallory's method for white fibrous connective tissue, **01,** was frequently employed.

For purposes of comparison and control, the silver method was applied to pieces from ten stages in the series. Beginning at 10 millimeters, the first six of these stages were in the identical order of the series; the remaining were at greater intervals. I was unable to get the silver method to succeed with specimens below 10 millimeters in length. Both the "rapid Golgi method" and the application of silver to material preserved in formalin were used. After considerable manipulation, a successful precipitation of the silver salt was obtained in all the stages above 10 millimeters deemed necessary, and in the younger of these especially the results were remarkably satisfactory.

Pieces of the spinal cord from ten of the stages were also subjected to the digestive action of pancreatin. The youngest of these measured 15 millimeters, the oldest fœtus was 28 centimeters, while the last two were from the suckling pig and from the adult. Pieces of adult human spinal cord were also subjected to the digestion experiments. The procedure followed in these experiments was that given by Flint, **02,** which had previously proven highly successful with pieces of other tissues. With the youngest specimens it was found necessary to remove the one or two segments required *in situ* and digest with the vertebral canal intact. Alone, they are so small and become so friable that there is danger of losing them entire. Older than 20 centimeters, if care be used, sections of the cord 2 to 3 millimeters in thickness may be handled during digestion.

In determining the period at which medullation begins, osmic acid was employed upon pieces taken from pigs between 14 and 25 centimeters.

By the Benda neuroglia stain, the neuroglia fibers and the chromatin of the nuclei stain deep blue. In fact, these are the only structures

which do stain blue. That the neuroglia fibers so stain is the chief means by which they are distinguished as such. In other words, the usual descriptions of neuroglia only apply to such fibers as are capable of being thus differentiated by the special stains, though it is perhaps true that only the fully developed fibers or those of a certain chemical nature give the blue reaction. Other forms of the non-nervous tissue of the spinal cord are stained brownish-red, or different shades of pink, by the sodium sulf-alizarate employed in the method.

In the earlier stages of the pig, before the neuroglia fibers are developed, the nuclei alone are stained by the blue. The general spongioblastic tissue stains deep enough pink for its structure and arrangement to be studied with ease, but the alizarin is not to be trusted to bring out cell boundaries when present. Therefore, in the earlier stages, other methods were used also. For the adult neuroglia, however, the Benda method exceeds all others for clearness of detail of the neuroglia fibers and for sharpness of contrast.

The Formation, Early Growth and Primitive Form of the Syncytium.

It is unquestioned that in the earliest stages of the embryo, the central nervous system is at first composed of individual cells, distinctly outlined and definitely arranged. At this stage His, **87**, states that the cells are neither connected with themselves nor with the periphery. That at about the time of the closing of the neural tube, the membranes of these cells begin to disappear and their cytoplasm becomes fused into a more or less common mass, has been shown by several investigators of this stage of the growth of the nervous system. His's papers of **83**, **86**, **87** and **89** often refer to the fusion, and in the illustrations certain of the conditions resulting from it are shown. Schaper, **97**, and others of those investigating the mitosis and distribution of the cells of the early nervous system also show these conditions.

The general name of *neurospongium* or *myelospongium* has been applied to this early form in the central system, but, after studying its formation and the changes it undergoes in the later development, it seems to me that the term *syncytium*, as used in other cases, is more expressive of the nature and behavior of the substance resulting from the fusion of the cells. I have found in the literature no description distinctly considering it as a syncytium and no account of the modifications it undergoes in the development of the adult form of the supporting tissue of the central nervous system.

Figs. 1 to 4 are given to show the beginning and primitive form of the syncytium. Being unable to obtain a pig embryo in the stage either before or at the closing of the neural tube, Fig. 1 is a copy of a drawing from His, 89, and represents the medullary plate of a rabbit embryo just before the closing of the tube. All the other figures are from my series of pig embryos.

Fig. 1 shows the wall of the nervous system when it consists of but one layer of cells. These are distinctly outlined with their boundaries intact. The nuclei are so placed that on the dorsal side, or what will be the ventricle after the closure, there appears a zone of cytoplasm (*a*) thicker than at the periphery (*m*). All the nuclei in mitosis (*g*), or germinal cells of His, are situated in the wider or ventricular zone. Neither of the limiting membranes are as yet evident.

Fig. 2 represents a stage (5 millimeter pig) after the tube has closed and after considerable cell-division has occurred in the walls of the tube. The nuclei are irregularly distributed in at least three rows. All nuclei showing karyokinesis (*g*) are situated in the ventricular zone (*a*). Throughout the section cell-membranes are rapidly disappearing, except those of the cells immediately bordering the ventricle. The membranes of the long axes of the cells persist longer than at the ends. The obliteration of the boundaries of the ends of the cells results in radially arranged, nucleated columns of protoplasm (*r*), extending from the ventricle to the periphery. Throughout the protoplasm of the section a general spongioplasmic network is easily seen. It is not interrupted along lines where one would judge cell-membranes have recently existed, and its filaments are somewhat coarser than one would expect from the study of other cells.

While the general epithelial character is still maintained at this stage, it is noticeable even here that the nuclei are becoming so arranged as to give the appearance of three zones or layers in the section:—an inner zone (*a*) practically free from nuclei other than those in the phases of mitosis; a middle, nucleated layer, and an outer layer (*m*), into which nuclei do not extend. It seems that the absence of nuclei in both the inner and outer layers is due to the nuclei of the cells forming the layers being situated in the ends of the cells farthest away from the inner and outer surfaces of the specimen. This three-layered appearance is maintained in the later forms by the migration of the nuclei from the inner layer, where they originate, into the middle nucleated layer, where they add to its thickness. One of the "germinal cells" (*g*, Fig. 2) is probably beginning to migrate.

The internal limiting membrane appears before the external. It is

evident in pigs of 5 millimeters (*mli,* Fig. 2). A study of their formation lends the impression that both of these membranes result from first a fusion and then a narrow condensation of the protoplasm immediately bordering the surfaces of the tube. The mesenchymal tissue surrounding the tube is already in the state of a completely formed syncytium, though the formation of the embryonic meninges from it is as yet scarcely begun.

Fig. 3 represents a lateral segment of a transverse section of the spinal cord of a pig of 7 millimeters (practically unflexed). The arrangement into three layers is more evident, though the middle, nucleated layer is much thicker than in Fig. 2. The formation of the syncytium is now almost complete. No cell boundaries are evident, except in the inner layer (*a*), which is nothing more than the remaining inner limbs of the cells bounding the ventricle. As in Fig. 2, this layer contains no nuclei other than those in phases of karyokinesis. The middle, nucleated layer is thickened, and its nuclei show a radial elongation. The syncytium once formed, there must be less resistance to the radial migration of the nuclei from their layer of origin. In fact, the very movements of the nuclei perhaps aid in producing the syncytium. The movements evidently play a rôle in its later arrangement.

The spongioplasmic reticulum is much coarser than in Fig. 2, showing both coarser and larger meshes. The cell boundaries having disappeared from the unexposed ends of the cells of the ventricular layer, the reticulum of these cells is continuous with that of the general syncytium of the section. The radially arranged columns apparent in Fig. 2 (*r*), if represented in Fig. 3 at all, have undergone great attenuation and appear drawn out into axial threads of more densely accumulated protoplasm (*r,* Fig. 3), in which several nuclei may be interposed. These axial threads seem to result from a spinning out of the reticulated protoplasm, due to the direction of growth and the movements of the nuclei through it. Thus radially arranged, the threads remain intimately connected with each other by means of numerous finer filaments, and the whole go to form a general reticulated syncytium with radially elongated meshes. At one or either end of the nuclei there is naturally more of the protoplasm than in the general diameter of the threads. This, if the extent of the threads is not realized, may give the appearance of conical or fusiform cells scattered through the section. That the axial threads seldom appear continuous through the nuclear layer is no doubt largely due to their intermingling among themselves instead of maintaining a straight radial course and, consequently, in the neces-

sarily thin sections, they do not appear throughout. However, many of the nuclei are not interposed in these threads at all, but are merely enmeshed in the finer reticulum. This is especially true for the more scattered nuclei situated in the outer portion of the nucleated layer.

The outer, non-nucleated layer (*m*) is beginning to assume the form which later characterizes it as the mantle layer or *Randschleier* of His. From the fine reticulum in Fig. 2, its protoplasm is now arranged into reticulated partitions bounding larger meshes, or in other words, it has become a sort of reticulated reticulum, a net seemingly with irregular areas of broken meshes. At the periphery the fine-meshed reticulum is more intact and a portion of it is condensed to form the now distinct membrana limitans externa (*mle*).

The connective-tissue syncytium immediately surrounding the neural tube (*p*) is at this stage becoming arranged with its meshes parallel to the external limiting membrane. Its anastomosing fusiform and stellate masses (cells) are becoming stretched upon the surface of the tube, due perhaps entirely to the growth of the latter, and thus there results the first appearance of the membrana meningea. At this stage no blood-vessels have grown into the spinal cord, and of course there are no nerve fibers.

Fig. 4 represents a ventrolateral segment from a transverse section of the neural tube of a pig embryo measuring 10 millimeters (flexed). The conditions found in this stage are easily seen to be transition forms of those shown in Fig. 3. Both of the limiting membranes have gained in thickness and the general radial arrangement of the syncytium between them is more marked than at 7 millimeters.

On close examination of the inner zone (*a*) what, under low power and especially in thicker sections, appears to be a layer of narrow cells, is really but the now evident ventricular ends of the axial threads formed by a spinning out of the protoplasm in the inner limbs of cells which were more or less distinctly bounded by membranes in Fig. 3, but which membranes have now entirely disappeared. The drawing out has resulted in the collapse of the original fine-meshed reticulum here, and now all that remains of it is represented by the fine lateral branches connecting the axial masses. As before, all divisions of nuclei occur in this zone and from the continued migrations, the middle, nucleated layer has gained further in thickness.

The further drawing out of the syncytial protoplasm gives rise to a more marked and coarser radial arrangement in the nucleated layer and to an interesting modification of the non-nucleated mantle layer (*m*). At the outer margin of the nucleated layer (*b*) the general

arrangement of the nuclei is disturbed, the nuclei being less oval and some of them may even lie transverse to the general disposition. This variation is coincident with a change in the behavior of the axial masses as they are continued into the mantle layer—a change which becomes more pronounced in the later stages. The axial masses, on reaching the border line between the nuclear layer and the non-nucleated mantle, apparently bifurcate, and the bifurcations give rise to a more tangled complex of the resulting threads. In the later stages it will be seen that some of the threads may even course at right angles to the general arrangement (Figs. 7 to 11). The tangle thus produced in this locality apparently becomes resistant enough to prevent, for a time, the nuclei from migrating into the mantle layer.

While the bifurcations of the radially disposed threads may be looked upon as a means of compensating the naturally greater dispersion of the substance at the periphery of the tube, yet they do not arise by a splitting of the threads as one might suppose from their appearance in the later stages (see Fig. 7). The bifurcations arise in practically the same way as the threads themselves. The method of their origin may be determined by comparing the structure of the mantle layer (*m*) in Figs. 2, 3, 4 and 7. The fine-threaded, small-meshed reticulum of the earlier stages grows into a coarser, larger-meshed form. Then there appear numerous, still larger meshes in the reticulum, and the smaller-meshed arrangement bounding these larger openings gives the appearance of irregularly arranged reticulated trabeculæ (Fig. 3). As the wall of the neural tube further grows in thickness, the mantle layer also thickens, and in the process undergoes a change in structure. The reticulated trabeculæ increase in substance, but, at the same time, being attached to the ever-extending periphery, they are drawn out or condensed till the meshes within them are obliterated and they become apparently solid trabeculæ with numerous thinner lateral branches extending, as before, into the large meshes (Fig. 4). Let this process of radial drawing out of the syncytium and condensation of the smaller meshes continue, there begins to be suggested a radial arrangement in the mantle layer itself (see figures of the later stages). Thus the radially arranged axial masses of the inner portion of the tube being continuous with the protoplasm of the mantle layer, their apparent bifurcations result in the course of the change in the form of the mantle layer.

Just as in the development of the nervous elements, the ventral portion of the neural tube precedes the dorsal portion (see Fig. 5), so, at first, the syncytial framework grows more rapidly in the ventral portion. It is on the ventral periphery that the mantle layer first thickens

and assumes the form shown in Fig. 4. Secondarily, both the nucleated layer and the mantle layer thicken in the dorsal portion also. As the wall of the tube increases in thickness, the syncytial protoplasm, being continuous throughout and common with both the internal and external limiting membranes, suffers a further spinning out. In other words, the axial threads, though gaining in the actual amount of their substance, are drawn out to greater individuality and more complete radial arrangement. In the radial direction of the growth, the coarse meshwork of the mantle layer, as shown in Fig. 4, is so drawn out that its meshes become radially elongated. In the process of elongation the filaments of many of the smaller meshes become adjacent and fuse with the larger threads, thus obliterating the smaller meshes and thickening the threads perpendicular to the limiting membrane. In this way the radial arrangement of the mantle layer is accentuated by a mechanical addition to certain of its threads, the smaller lateral threads connecting them appearing less significant by contrast, and the apparent bifurcation of the radial axes of the nucleated layer becomes more pronounced.

The general radial arrangement of the syncytium thus produced is maintained up to pigs of 7 centimeters, when it is destroyed in a way to be described later. In pigs of 3 to 5 centimeters the *membrana limitans externa* becomes less distinct. It either decreases from being spun out into the general framework, or the syncytium of the mantle layer so increases in density as to destroy the contrast.

In the literature the radial axes, etc., are almost exclusively described as cell-processes, and the nuclei nearest the ventricle (ependymal cells) are given all the credit for the entire framework. Hannover, **44,** and Stilling, **59,** were, I think, among the first to describe them as such. Though primarily dealing with the development of the nervous elements, several of His' papers touch upon the subject. Of necessity he considered the early, coarse reticulum of the mantle layer as produced by the anastomosis of greatly extended and much branched peripheral outgrowths of the ependymal cells. Among the others, Weigert accepts this form of description and, quoting Sala y Pons, states that the ependymal cells send out two processes, one to the ventricle and one to the periphery. From the method of its origin, the nature of its structure, and from its later modifications, I do not think the term *cell-processes* adequately describes the framework.

Many of the investigators have based their conclusions solely upon appearances obtained with the silver method. In the first place, the silver salt will not differentiate the earliest stages, and in the second

place, when used alone upon the stages it does act upon, it cannot be trusted to tell the whole story. When it stains only the radial (thicker) threads, as it usually does in the stages before these are broken up, the picture obtained is highly suggestive of cell-processes, especially if the precipitation involves only the nuclei nearest the ventricle. Perhaps, because of incomplete precipitation of the silver salt, few observers have called attention to the smaller lateral branches or filaments uniting the radially arranged "processes." Lenhossék, **91**, notes such for the human embryo, but found them only at the beginning of the processes. Retzius calls attention to abundant "*Moosartige Aestchen,*" and Kolster, **98**, describes especially strong lateral branches in the embryo salmon. Even if seen very abundant and uniting the "cell-processes," none see in these lateral filaments merely a modification of an earlier condition of a continuous framework. In a comparative study of the ependyma, including quite a number of animal species, Studnicka, **00**, with other methods than the silver, describes and pictures "ependymal cells" continuous with each other by numerous heavy connections which he calls intercellular bridges. In some forms he especially describes these as occurring near the ventricle or in the region of the nucleus of the ependymal cell. His pictures show a perfect syncytium. Gierke, **85** and **86**, though working with very inferior methods, in his illustrations of the early stages of the *markgerüst* shows a syncytium, though he describes it in terms of anastomosing cells.

The Proliferation, Migration and Distribution of the Nuclei.

From the earliest stage, in which the embryonic spinal cord consists of a single layer of cells, until even after the shape of the gray figure is assumed, the nuclei show evidences of continual migration and change of position. The general direction of the migration is radially outward from the more thickly nucleated region nearer the ventricle or central canal. The mantle layer, appearing early and seemingly as a peripheral excrescence of the rapidly increasing syncytial protoplasm, is at first non-nucleated. In pigs of about 10 millimeters, the stage when blood capillaries begin to grow in, a few nuclei may be found in the mantle layer, but up to 5 centimeters all such belong exclusively to the blood capillaries and are therefore acquired from the outside. Up to 5 centimeters in length the migration of the nuclei peripheralward is apparently checked at the inner border-line of the mantle layer by the greater complexity there in the arrangement of the filaments of the fibrillated protoplasm (*b*, Figs. 4, 7 and 9). Later, as the early arrangement is broken

up by the further extension of the periphery, and by the development, ingrowth and further elaboration of the nerve-elements and blood-vessels, nuclei invade the mantle layer both from the nucleated layer (ectodermal) as well as from the outside (mesodermal). The latter nuclei may either accompany the walls of the ever-extending blood-vessels or as independent ingrowths of the developing pia mater. This double invasion goes on till, in pigs of 8 to 10 centimeters, the mantle layer appears almost as thickly nucleated as any other part of the section. The nuclei are later dispersed in the final rapid thickening of the mantle layer in consequence of the medullation of the axones coursing in it.

Only at first is the origin of all the nuclei of the section strictly ectodermal, though at all times perhaps a good majority of them are of this origin. In all the earlier stages the mitoses giving rise to the nuclei of this origin all occur in the inner zone of the section, or the most ventricular portion of the wall of the neural tube and just within the *membrana limitans interna.* All the divisions are by the indirect method, and in almost every case the long axis of the polar spindle lies parallel to the membrane or transverse to the general radial structure of the tube. In the process the nuclei become much swollen and bubble-like and, in the middle phases, the chromatin filaments are surrounded by an area of less stainable substance—a clear court. In the early stages, when the cell-membranes are more or less evident (Figs. 1 and 2), the phases of division involving the membrane may be observed. In the later stages there are no real cell-membranes. What at first appear as such, when followed through the phases, seem nothing more than a zone of the more stainable protoplasm packed away from the center of activity during the swelling of the nucleus (Figs. 3 and 4). This resemblance to a cell membrane is exactly similar to that which appears about dividing nuclei in the connective-tissue syncytium outside the central nervous system, where individual cell-membranes are impossible (see Fig. 5).

After division, the nuclear membrane reforms and at least one product of the division begins to migrate toward the periphery. In the process of migration the nucleus becomes slightly elongated and, as it leaves the limiting membrane, the clear court it occupies usually becomes pointed and then gradually disappears and, accompanying its collapse, the surrounding protoplasm is radially drawn out and, usually by means of it, the nucleus, for a time at least, remains in direct connection with the internal limiting membrane. A great majority of these nuclei, in the further migration, become so dispersed as not to appear interposed in the radial filaments but are merely enmeshed in the less accumulated portion of the syncytium between the filaments.

Altman, **81,** was, I think, the first to call attention to the locality in which the mitoses occur. He thought that all divisions take place within the zone immediately bordering the ventricle—the ependymal layer. This view was supported by His, **87,** as true for human and rabbit embryos. Rauber, **82,** using a series of frog embryos, denied a special seat of cell-division, having found mitotic nuclei in both the ependymal and outer layers. He explained the contradiction as due to others having taken material at different stages of development, admitting that in the very early stages all mitoses are ventricular. Merk, **86,** and Vignal, **89,** admit that cell-division may occur in other regions than the ependymal layer. The latter, however, did not actually observe any elsewhere but assumed their occurrence from a second assumption that the nuclei increase more rapidly than could be accounted for by the ventricular mitoses observed. Schaper, **97,** in his studies of the early differential processes in the embryonic nervous systems of various species, touches upon this point and reaches a conclusion which, from my study of pig material, I am convinced is correct. He observed that it is only in very young embryos that all the mitoses occur in the ependymal layer; that after the blood-vessels have entered the central system and begin to elaborate, extra-ependymal mitoses may be found, and that therefore the distribution of mitotic nuclei is largely a question of proximity to nourishment. In the early stages, the ependymal zone may also be considered a locality presenting less mechanical resistance to the phenomenon. Hamilton, **01,** quotes Schaper and practically accepts his conclusions as true for the white rat. Paton, **00,** investigating the histogenesis of the cerebral cortex of the pig, finds most of the dividing cells in the ependymal layer and very few in the mantle layer. In my sections of the spinal cords of pig embryos nearly all the extra-ependymal mitoses are found in the middle nucleated layer, which itself results from the migration of nuclei from the ependymal layer, and which is distinguished, on the one hand, by having its nuclei less thick than in the ependymal layer, and on the other, by the fact that the adjacent mantle layer proper is relatively free from nuclei (see Figs. 5 to 8). While the middle layer begins to appear quite early, it never presents mitotic nuclei till sometime after the blood capillaries are acquired.

The following observations may convey some idea of the rate at which division occurs in the spinal cord of pig embryos and the relative numbers in the ependymal and extra-ependymal layers:

At 5 millimeters (unflexed) all mitoses are not only in the ependymal layer but in the ventricular surface of that layer. Counts of twenty sections of 5μ each give an average of 5 mitoses per section.

At 7 millimeters (unflexed) mitoses are more abundant, but all are in the ependymal layer. They are more numerous in the ventral than in the dorsal portion of the section and often occur embedded in the layer, not touching its ventricular border. Counts in twenty sections of 5μ give an average of 16 mitoses per section.

At 10 millimeters dividing nuclei are still more numerous. Now and then evidence of blood capillaries may be seen, and, very occasionally, an extra-ependymal mitosis. Average mitoses in twenty sections, 24 per section.

In the spinal cords of pigs of 13, 15, 17 and 20 millimeters, a computation gave an average of about 20 mitoses per section. Of these about one dividing nucleus per section occurred in the middle nucleated layer. Blood capillaries increase rapidly in abundance with the size of the specimen and occasional mitoses may be observed in their walls. These were not taken into account.

At 25 millimeters the number of mitoses begins to decrease appreciably, the decrease occurring entirely in the ependymal layer, and in pigs of 35 millimeters mitoses in this layer are but seldom observed. From 4 centimeters on there are practically no divisions to be found save what is apparently either a leucocyte or at least a nucleus of mesodermal origin.

In pigs of 25 millimeters, transverse sections of the spinal cord begin to suggest the characteristic shape of the adult. The more rapid lateral growth begins to result in the ventral and dorsal median fissures, and the nuclei begin to be so arranged that the form of the gray figure may be distinguished. At 30 millimeters the central canal, which hitherto has maintained the relative proportions of a ventricle, suffers a collapse of its dorsal two-thirds and the remaining, ventral third begins to assume the circular form (compare Fig. 8 with Fig. 6) and the ependyma to appear under low power as a single layer of ciliated epithelium. Merk, **86**, makes the statement that in both birds and mammals nuclear division ceases when the central canal has become circular, and the ependyma ciliated. I find that in the pig, however, evidences of cilia appearing out of the internal limiting membrane as early as 10 millimeters.

The above observations agree in the main with what others have found in embryos of other species, namely, that karyokinetic activity increases in the earliest stages, reaches a period of maximum activity which is maintained for a time, and then declines, and finally, at a comparatively early stage, the division of nuclei of ectodermal origin ceases altogether. Both the period of maximum activity and the time of cessation varies for different animals. Hamilton, **01**, thinks that in the brain of the rat

the maximum period occurs after birth, while numerous observations upon other mammals, chiefly those born much more mature than the rat, are to the effect that both the maximum period and the period of cessation occur long before birth.

In the migration from the ependymal layer, the anlage of the ventral horn is first to appear. It begins as an area of loosely arranged nuclei on the ventrolateral aspect of the ependymal layer (*vh*, Fig. 5). The whole ventrolateral half of the ependymal layer contributes to its formation. At 15 millimeters, the migration from the dorsolateral half of the ependymal layer has become more active, and as a result the anlage of the dorsal horn may even exceed that of the ventral in width (Fig. 6). Then follows a general thinning of the ependymal layer, which the hitherto abundant mitosis has maintained quite thick, till at 30 millimeters (Fig. 8), mitosis having practically ceased, the layer becomes an ependyma similar to the adult form.

The period of differentiation of the growing tissue elements into those which will produce neurones and those that will take part in the formation of the neuroglia is difficult to determine. Certainly it may be said of my preparations that in pigs up to 15 millimeters in length evidences of differentiation are little more than theoretical. With the exception of those nuclei in the phases of mitosis, all the nuclei of the spinal cord, both in the ependymal layer and in the middle nucleated layer, are so nearly identical in structure, size and general appearance that an attempt to classify them on the basis of differentiation is impossible. They are all of the large vesicular type, and the protoplasm about them never occurs in definite form and amount, never shows definite outline, and never stains differently from the general syncytium in which the nuclei are embedded. Numerous nuclei, especially those in the ependymal layer, show tapering masses of more densely accumulated protoplasm at their either end, but these masses may be usually observed as continuous with the less compact protoplasm between them. In the ependymal layer these masses are portions of the radiating axes of the syncytium (Figs. 4, 6 and 7). In the less densely nucleated middle layer they have sometimes a stellate and often a fusiform appearance, and still again the protoplasm may be present at only one end of the nucleus (Figs. 6 to 9). In all cases in the early stages these appearances are considered as resulting from the radial drawing out of the protoplasm consequent to the later growth of the specimen and the radial direction of the migration of the nuclei.

Not till pigs of 15 millimeters do my preparations show anything in the spinal cord characteristic of the neurone, although as early as 7

millimeters the embryonic connective tissue surrounding the neural tube shows parallel arrangement indicative of the future paths of the dorsal and ventral roots (Fig. 5). First, there appears in the ventrolateral aspect of the middle layer an occasional nucleus somewhat larger than its neighbors and whose chromatin shows a tendency to collect into one larger mass or single nucleolus. At this time the surrounding protoplasm displays no differentiation from the general. At 20 millimeters these nuclei are more abundant. Ordinary stains show no difference in the protoplasm about them, but the silver method at rare intervals gives pictures suggestive of the ventral-horn type of neurone. At 30 millimeters there is usually a group of these nuclei displayed in the ventrolateral region (*n*, Fig. 8). With ordinary stains they begin to show a differentiated cytoplasm about them and the silver method gives a few characteristic nerve cells. In the anlagen of the spinal ganglia the changes in the nuclei and the development of the neurone protoplasm precede those in the spinal cord by at least 2 millimeters; that is, here the changes begin to appear in pigs of 13 millimeters instead of 15 millimeters, as in the spinal cord.

It may be said in passing, that in none of my preparations have I been able to observe evidences supporting the view of either Fragnito, 02, or Kronthal, 02, as to the formation of the cytoplasm of the neurone. After describing the changes of the nuclei into the nerve-cell type—"primary nuclei"—Fragnito holds that these primary nuclei, at first free from cytoplasm, become surrounded by the general smaller or "secondary nuclei," which so arrange themselves and then undergo such changes as to give rise to the cytoplasm and Nissl bodies of the neurone, including the axone and dendrites. Kronthal ascribes a somewhat similar office to wandering leucocytes.

The title of this paper bars an attempt to discuss the processes by which the neurone develops. The point in mind is whether the products of the mitoses in the neural tube are not at first totally indifferent, capable of developing into either neurones or neuroglia. For a long time the impression was obtained, chiefly from the investigations of His, that the neurone develops from the product (neuroblast) arising from the mitosis (germinal cell of His) in the early ependymal layer, and that the embryonic neuroglia (spongioblasts) arise as a transformation of the ependymal ("epithelial") cells. Contrary to this idea, in 1889, it was suggested by Vignal that not only the germinal cells but all the cells of the neural tube are indifferent up to the time when grouping is manifested in the ventral horns. Later, Schaper, 97, investigated the question more carefully and reached the conclusion that His'

germinal cells are none other than undifferentiated dividing elements, that the products of their division may develop either into neurones or neuroglia, or may further divide and still be indifferent. In other words, both the neuroblasts and spongioblasts are derived from the germinal cells. Paton, 00, working with pig material, agrees with Schaper in this assertion. In one of his later discussions, His, 01, refers to Schaper's paper and, while not wholly admitting his position, calls attention to the general definition of "germinal cell": A young element in a state of division, as yet morphologically undifferentiated (*in status nascens*), which by its globular form and bubble-like appearance is easily distinguished from its surroundings.

In the spinal cord of the pig it is true that up to a certain stage all the products of the mitoses are so nearly identical that, morphologically, no trustworthy distinctions can be made among them. On the other hand, however, it may not be just to judge them indifferent upon wholly morphological grounds. A neurone, for example, to be distinguished as such, must first acquire certain characteristic features and, while these features are certainly acquired by elements seemingly similar in every respect to those which acquire other features, it is difficult to say whether they do not possess, from the first, the peculiar properties necessary for the acquirement, or whether such properties result in reactions to influences of later environment. It is, I think, certain that collectively the dividing nuclei known as germinal cells give rise to both embryonic neuroglia and to nervous elements, and with the ordinary technique one can but agree with Schaper that the products of the divisions are at first indifferent.

Paton, 00, finds in the brains of embryo pigs, except in the very earliest stages, two types of germinal cells; one large, having protoplasm, and the other smaller with no protoplasm about it. Both of these he says give rise to indifferent elements. Hamilton, 01, also describes large and small types in the white rat, and ascribes to the larger the origin of neurones and to the smaller the origin of neuroglia. In my preparations of the spinal cord of pig embryos, it seems to me that the amount of protoplasm about a dividing nucleus and, indeed, the apparent size of the nucleus itself, depends upon its position in the syncytium. If the dividing nucleus occurs in the ventricular border of the ependymal layer where the absence of nuclei allows a greater relative abundance of protoplasm, it will of necessity be enclosed by a greater amount. If the division occurs among the very compactly arranged nuclei of the ependymal layer, which is thick during the active period of mitosis, the nucleus will be surrounded by much less protoplasm, or often apparently

none. Fitting in among the other nuclei, it may be compressed in the opposite plane to that of the section and may therefore, in section, appear smaller than others (see Fig. 5). If the germinal cells occur extra-ependymal (which in pigs they very rarely do), just as the more scattered non-dividing nuclei there, they may have a varying amount of the more compact protoplasm about them, or they may have none at all, and, owing to the plane of section, they may appear large or small. After the blood-vessels have grown in, some extra-ependymal mitoses may be of mesodermal elements rather than of those in question.

As before suggested, after the first entrance of blood capillaries, the framework of the central nervous system is no longer solely of ectodermal origin. In addition to what is contributed by the walls of the blood-vessels, the pia mater itself, which begins to take form at an early stage, sends numerous ingrowths into the spinal cord. In pigs of 20 millimeters these ingrowths may be discerned in sections, and at 30 millimeters they are more marked (*i*, Figs. 8 and 9), and are sometimes accompanied by nuclei from the mesodermal tissue of the pia. In the later stages, after the development of the fibers of the white fibrous tissue which occurs long before neuroglia fibers are differentiated, the mesodermal ingrowths are easily seen in section stained by Mallory's method for white fibrous tissue. By far the greater contribution of mesodermal tissue, however, is brought into the central nervous system by way of the blood-vessels. The capillaries, first entering in pigs from 9 to 10 millimeters, carry in this tissue both as composing their walls and their contents. As they branch and ramify, their walls thicken and send processes into the surrounding ectodermal tissue. These processes, just as those from the pia direct, are accompanied by nuclei of mesodermal origin. Further, leucocytes have been observed passing through the walls of the capillaries to wander into the tissue without. Thus, if with this point in view, the changes are carefully followed into the later stages, one is convinced that nuclei from these two sources constitute an appreciable quota of those present in a section of the spinal cord. The mantle layer (Randschleier), at first thin and almost free from nuclei, gradually thickens and gains nuclei (Figs. 6 to 10), and up to 80 millimeters, at least a majority of the nuclei situated in it, whether in mitosis or not, may be considered as mesodermal nuclei acquired in the above manner.

Attention has recently been called to these mesoblastic constituents of the central nervous system. In a joint paper, Capobianco and Fragnito, **98**, noted the manner of their ingrowth, migration and distribution among the ectodermal elements. Later Capobianco, **02**, attributes to

these mesodermal elements the capability of taking part in the development of the neuroglia. In addition, Hatai, **02,** observes in white rats dividing cells of the endothelium of the capillary walls and states that some of the cells resulting from these divisions migrate into the surrounding tissue. He thinks, further, that these migrating endothelial cells become neuroglia cells.

Thus it may be assumed from the above that there become distributed in the central nervous system, in addition to the nuclei belonging to the capillary wall proper, three forms of elements of mesodermal origin: (1) Leucocytes or wandering cells, which usually but not necessarily enter by way of the blood-vessels; (2) endothelial cells from the *intima* of the capillaries; (3) nuclei belonging distinctively to the connective tissue proper, which enters either as ingrowths of the developing pia mater or secondarily from the *externa* of the blood-vessels.

The assertion that these mesoblastic elements take part in the formation of the neuroglia is, I think, not wholly warranted but, at the same time, it is difficult to refute it. The difficulty lies chiefly in the fact that the mesodermal elements begin to enter at a time when no fibers are differentiated and there is no way to characterize the connective-tissue syncytium itself, and the consequent intermixing and fusing of the syncytia from the two sources renders it well-nigh impossible, especially in the outer layers of the specimen, to distinguish all the elements of mesodermal origin from those which are not. In his study of the formation of the connective tissue of the body outside the central nervous system, Maximow, **02,** describes three forms of elements which are perhaps identical with those mentioned above as contributed to the central nervous system. He describes the leucocyte as the ordinary polymorphonuclear variety and, in addition to the functions ascribed to it as such, thinks it may change into other forms. The endothelial cells he speaks of as "polyblasts" and, after discussing whether they can be considered as really of the endothelium, he describes them, after their migration from the capillaries, as similar to lymphocytes and as actively wandering and phagocytotic. The third form is the "fibroblast," the pre-existing connective-tissue corpuscle, directly concerned in the formation of white fibrous connective-tissue. Maximow's observations are cited in order to suggest the probability that the mesodermal elements in question may play the same rôles within the central nervous system as they do outside it, that is, take part in the formation of connective tissue proper. However, the embryonic connective-tissue elements may be considered highly responsive. To all appearances, very similar in the early embryos, they later become so separately differentiated that some produce white or

elastic fibrous tissue, some cartilage, etc. Within the central nervous system and subjected to the environment there, some may contribute to the formation of neuroglia.

If the term "neuroglia" includes the entire framework supporting the central nervous elements, then of course mesodermal elements contribute to it. But, such of the supporting tissue as can be distinctly designated as having mesodermal origin is of the white fibrous variety. Sections of the adult spinal cord prepared by a method differential for white fibrous tissue show abundant ramifications of this tissue. If, on the other hand, the term "neuroglia" includes only that portion of the framework which is differentiated by the special neuroglia stains, then it becomes difficult to say, from my preparations of pig embryos, whether any of the tissue is of mesodermal origin or not. Just as that portion of the framework, which is of undoubted ectodermal origin, early assumes and maintains the form of a syncytium, the same form of development has been conclusively shown (Spuler, **96**, Mall, **02**) for the connective tissues outside the central nervous system. And, as said before, the mesodermal ingrowths begin before fibers are developed in either syncytium, and the result is a fusion of the substances from the two sources with no means of determining where the one begins or the other leaves off. The nuclei of the two migrate and intermix and, with the exception of nerve-cell nuclei and those of the ependymal layer, it becomes impossible to tell the source of a nucleus by its appearance or position. All these nuclei have been often referred to collectively as neuroglia nuclei. While these so-called neuroglia nuclei begin to undergo variations long before neuroglia fibers begin to appear, yet for some time after the variations begin to appear in my preparations, for any type of nucleus found in the spinal cord a similar type may be distinguished in the embryonic connective tissue outside.

Neuroglia, to be distinguished as such, must possess those properties which characterize it, but these characteristics do not appear till after the intermixing of the material from the two germ layers. Morphologically, neuroglia fibers are similar to those of white fibrous tissue in some of its less compact arrangements. That neuroglia fibers differ in their microchemical properties from those of white fibrous tissue is the chief means by which the one may be distinguished from the other. By the special neuroglia stains (those of Weigert and Benda) the easily discernible ingrowths from the pia certainly do not give the stain reaction which characterizes neuroglia, and tests made of these methods upon various tissues (Hardesty, **02**), indicate that the methods give trustworthy differentiation. On the other hand, should any of the fibers of white

fibrous tissue in the central nervous system give the neuroglia reaction, by this reaction they would be classed as neuroglia fibers.

The Final Form of the Syncytium and the Development of the Neuroglia Fibers from it.

The syncytium is formed early, before the embryonic nervous system is invaded by ingrowths of mesodermal tissue, and thereafter is manifest in all stages. Its arrangement, however, changes as the specimen grows and acquires the form and structural components of the adult. Its substance being plastic, its variations are expressive of the processes of growth. At first, resulting from the fusion of radially arranged columnar cells, it soon assumes the form of a sort of filamentous reticulum continuous with the internal and external limiting membranes. Then, as the nuclei of the inner zone proliferate and migrate along radial lines, and as the external limiting membrane grows further away from the internal in the thickening of the wall of the tube, the syncytium assumes the form of radially arranged thicker filaments intimately continuous with each other by the more attenuated portion of the reticulum between them. As the specimen grows further, the radial filaments thicken and are further drawn out, and for a time, the radial arrangement becomes more marked. It is finally obliterated by the ingrowths and medullation of the neuraxes and the further structural changes toward the adult form. Then the syncytium, by its plasticity, assumes the shapes of the interspaces of the elements which it supports.

The "neuroglia nuclei" begin to show variations in pig embryos of 20 millimeters. Previous to this they are all of the large vesicular type. At 30 millimeters (Figs. 8 and 9), while the majority of the nuclei are still of the large vesicular variety, many may be seen undergoing changes which in all probability result in the various forms usually described in the adult tissue. The changes consist in a decrease in size and a more compact arrangement of the chromatin resulting in deeper staining. The smallest appear as blue-black spheres of less than half the diameter of the large vesicular form. At 30 millimeters, when the migration has resulted in the demarcation of the dorsal horn, the small nuclei are more abundant in the dorsal horn than elsewhere in the section.

Up to about 25 millimeters the ventricle increases in size; then, with the cessation of mitosis and the thinning of the ependymal layer, it decreases in size by a collapse of its dorsal two-thirds and a fusion of the internal limiting membrane along the mid-line (compare *d*, Fig. 6, with *sp*, Fig. 8). From 30 millimeters the ventricle continues to decrease,

but more slowly and always by a collapse of its dorsal portion, till at 70 millimeters a central canal results which is but slightly larger than the adult.

The mantle layer from its first appearance completely encompasses the ventral aspect of the specimen, while on the dorsal aspect the ependymal layer for a time extends to the very periphery (*d,* Fig. 6). With the collapse of the ventricle and the further lateral growth of the specimen, the mantle layer closes about the dorsal aspect also. Then, as the lateral growth of the embryonic spinal cord continues, depressions naturally result at both ends of the mid-line. Subsequently, the growth goes on in such a way that, as the depressions deepen, the one on the ventral aspect remains open as the anterior median fissure, while the dorsal one collapses almost as fast as it is formed, and becomes the posterior septum. What for a time is apparently a portion of the posterior septum extending through the nucleated layers, is only the remains of the internal limiting membrane (*ms,* Fig. 8). As the neuraxes grow in, this appearance is obliterated by the dorsal commissure, etc. The investing pial tissue accompanies the depressions and aids in maintaining the posterior septum of the adult.

No nerve axones are discernible in my sections of the spinal cord of pigs at 15 millimeters. At 25 millimeters neuraxes have begun to appear in transverse sections as fine dots embedded in the syncytium and staining like it. They are more evident in the mantle layer, especially in the dorsal portion. At 30 millimeters (Figs. 7 and 8) the syncytium of the mantle layer is more thickly studded with axones, and from 30 millimeters upward they become more abundant and more generally distributed and show a slight and gradual increase in size. Until the processes of medullation begin (16 to 20 centimeters), the ordinary methods show them simply as dots and staining only a darker shade of the same color as the syncytium which closely invests them. The silver method, of course, differentiates them clearly.

In the mantle layer the radial arrangement of the syncytium is practically perpendicular to the periphery except at the ventral aspect of the ventricle (*mv,* Figs. 6 to 9). Here the more rapid lateral direction in the growth of the wall of the neural tube results in the syncytium being drawn into an arrangement parallel to the periphery. However, as the lateral growth continues and the depression which results in the median fissure appears, the lateral tension decreases and becomes equalized and soon filaments may be seen arranged in both directions (*mv,* Figs. 8 and 9). A suggestion of this result can also be seen through the ventral portion of the middle nucleated layer (*mn,* Fig. 8).

A comparison of results obtained by the silver method with those obtained by other methods is interesting. The silver method can be considered little more than an aid in the interpretation of appearances obtained by the more general stains, and should always be used collaterally. Four of the figures given are combination drawings comparing appearances given by the Benda method with silhouettes resulting from the application of the silver method to spinal cords of the same respective stages. It is well known that the silver method often differentiates certain structures very clearly, but from time to time varies greatly in its selectiveness for reasons not well understood. Furthermore, being a precipitation method, the structures it does show are coated with the reduced salt and consequently are of unnatural size and coarseness. The amount of detail depends wholly upon the extent to which the salt is allowed to precipitate. Certain detail as to the external form may be obtained, or practically none at all, for the specimen may be so clogged as to appear as a black, indefinite mass. These facts have been especially impressed in my experiments with the method upon the spinal cords of embryo pigs. The external limiting membrane and, indeed, the whole mantle layer is often clogged beyond recognition of detail or outline, when the other layers are practically unaffected. Looking over the illustrations of others, one is convinced they experienced the same difficulties and the fact is further impressed of the folly of accepting, uncontrolled, the results of the silver method as giving either the whole or even the true story. The preparations from which the accompanying drawings were made are the result of considerable experimentation, and I think they show about all the method is capable of showing.

One seeming peculiarity of the silver method shown in the drawings is that in pigs up to 70 millimeters, the reduced salt shows a marked preference for only those nuclei situated in the ependymal layer. With the exception of an occasional nerve-cell when present, all other nuclei are unlocated. Fortunately only a small percentage of the ependymal nuclei are selected. In these young stages the necessarily short segment of the very tender spinal cord is usually left intact in the vertebral canal and the ependymal nuclei, being nearer the solution in the ventricle and connected with the internal limiting membrane by the heavier inner ends of the axial filaments, are perhaps the first to be reached by the silver solution. The potassium bichromate being already in the specimen, the surfaces of the nuclei may act something like nodal points or centers of crystallization of the resulting compound. The deposit once started upon a surface, it continues from that surface along the lines of least resistance, which here seem to be the radial axes of the syncytium or the

largest individual surface of the protoplasm connected with the nucleus. The precipitation is at first much less extensive upon the more attenuated collateral filaments connecting the radial axes, and fortunately so, for were these all covered, the sections, necessarily thick to show the entire course of the axes, would be clogged. The few of the connecting threads that are shown are usually shown for only a short extent.

By comparing Fig. 7 with Fig. 11 it appears, by the silver method, that the radial axes increase in size with the growth of the animal from 15 to 70 millimeters. Staining methods applied to these stages do not show so marked an increase in size. Also in Fig. 7 (15 millimeters) the precipitation of the reduced salt is evidently not so complete as in the other figures. Both of these variations may indicate a development of the selective property, for in pigs of less than 10 millimeters I have been unable to obtain a differential precipitation at all.

The bifurcations of the axial filaments, the formation of which has been already described, are very evident by the silver method but are barely suggested in the stained preparations. This is not wholly due to the difference in the thickness of the two sections represented in each drawing (80μ compared with 5μ), but is also due to the fact that the whole of the syncytium is shown in the thinner section and but a part of it in the silver preparation.

The greater complexity in the arrangement of the syncytial filaments along the boundary line between the nucleated and the mantle layer (*b*, see figures) is shown by both methods but more clearly by the silver. Its formation begins early, as shown in Figs. 3 and 4, but its increased complexity in the later stages (Figs. 7 to 10) more fully suggests that it may, for a time, prevent the nuclei from migrating into the mantle layer. At 7 centimeters (Fig. 11), axones are more abundant and the boundary line begins to be broken up, and the nuclei begin to invade the mantle layer rapidly, till at 8 and 9 centimeters, nuclei become almost as abundant in the mantle as in the middle layer.

At the point of bifurcation, where the filaments come together, there is a greater amount of the plastic substance than in the simple diameter of a filament. Both because of this and also due to the angles formed by the junction, there is usually a greater deposit of the silver salt at these points than elsewhere and the precipitation usually tends to run further out on the collateral filaments. There seems to be a progressive increase of this phenomenon from 15 millimeters upward. In pigs of 55 millimeters the points of bifurcation (*b*, Fig. 10), if isolated, would, to say the least, strongly resemble the "astroblasts" and "astrocytes" described by various authors (Reinke, Kölliker, Lenhossék, etc.). And

indeed such are often isolated either by section or by incomplete precipitation of the silver. In this way it is possible by the silver method to get "neuroglia cells" without nuclei. For example, in Fig. 11 the bodies designated by *c* in all probability contain nuclei, while those indicated by *b* do not. When the radial arrangement of the syncytium is broken up, these bodies, more abundant and more marked than in the earlier stages, naturally become isolated in the process. A further study of silver preparations of the later stages leads to the conclusion that, while most of these bodies usually described as neuroglia cells do contain nuclei, many of them do not.

One of the first evidences of the breaking up of the radial arrangement is the rupture and pulling away of the ends of certain of the axial filaments from their attachments. This beginning is shown in Fig. 11 (pig of 7 centimeters) where at least two of the filaments with their interposed nuclei (*e*) have lost their direct continuity with the internal limiting membrane, and in the mantle layer several seem broken away from the periphery. At the same time occasional nuclei (*c*), other than those of the ependymal layer, begin to be selected by the silver and the fine filaments immediately about them give the well-known figures.

The radial filaments have been frequently described but usually as processes of ependyma cells. Lenhossék, **95**, thinks their breaking away from both the central canal and from the periphery is the result of their contraction. Leaving aside their manner of origin, if the ependymal nuclei with their common protoplasm can be considered as cells, then for a time the radial filaments, after the silver method, do resemble processes, but processes continuous with each other by means of numerous smaller filaments between them. The rupture of the axial filaments, is, I think, more probably due to unequal growth processes than to their contraction. At the time the rupture occurs the wall of the neural tube is rapidly thickening by the ingrowth of new nerve-elements, by the enlargement of those already there, and by the increase and extension of the blood-vessels, and the resulting tension is probably greater than the rate of growth of the filaments.

With the further enlargement of the specimen, the ingrowth, arrangement, and elaboration of the neurones and blood-vessels, the obliteration of the radial arrangement continues, till, as is well known, a state is reached in which no vestige of the radial arrangement remains save in the then thin ependymal layer immediately surrounding the central canal. Here such an arrangement is maintained even in the adult. This arrangement broken up, the syncytium, by the ordinary staining methods, is even more apparent than before and consists of a continuous,

plastic, nucleated mass in which the other elements of the nervous system are embedded. The shape of a given portion of the mass (cell) depends upon the shape of the space it occupies. The characteristic *Deiters' cells* are to be observed only after the medullation of the axones has resulted in interspaces giving them their shape. The pictures of Deiters' cells obtained by the silver method (which, however, was not employed in their original description) can be considered as the result of a deposit usually beginning on the nucleus, clogging the mass about it, and extending variable distances along the filaments and trabeculæ (processes) connecting the mass with its neighbors. The earlier possibilities of such appearances are shown in Fig. 12. This figure is taken from transverse sections of the spinal cord of a fœtal pig of 20 centimeters. The processes of medullation are underway. The larger portion of the drawing represents conditions shown by the Benda neuroglia stain and accompanying this are three masses chosen from a silver preparation of the same specimen. Either of the masses designated by *a* can, I think, be correlated with silver picture *a'*. The picture *b'* can be looked upon as a deposit of the silver upon a mass similar to that indicated by *b*. With a little more clogging at the center this would give a neuroglia cell without a nucleus. Masses similar to *x* can be easily imagined in the section.

Many appearances to be observed in the pig are repeatedly described in the literature employing the silver method. Also, for example, Gierke, **85** and **86**, who worked before the silver method was in general use, described glia cells, nucleated and non-nucleated, and pictures them with anastomosing processes. Some of his drawings are easily correlated with the usual silver pictures.

The size of a silver picture and the length and density of its processes depend both upon the size of the mass and the extent of the precipitation. The "processes" are seen to radiate in all directions, for the reason that the sections in which they are seen are usually thick enough to allow considerable perspective. Very long and finely attenuated processes are more frequent after fibers are developed in the syncytium, and many such processes no doubt represent fibers.

The development of the neuroglia fibers from the syncytium is a process of transformation.

Fibers are first differentiated in the spinal cord of pigs from 16 to 20 centimeters. This is not till after the processes of medullation have begun. Fibrillated areas and what appear as fibers occur in the syncytium before this time, but by the Benda method they stain a light brownish-red like the general syncytium instead of the deep blue characteristic of neuroglia fibers. Therefore, by definition they cannot be con-

sidered neuroglia fibers or, at least, not as adult neuroglia fibers. They may develop their chemical difference later. On the other hand, some of them may be threads of the white fibrous connective tissue which are supposed never to color as neuroglia fibers.

In Fig. 12 is shown an area containing fibers, some of which are beginning to develop their chemical difference from their surroundings. Fig. 13 is from a suckling pig of about two weeks. The following are some of the steps in the transformation indicated.

After the medullation has begun, the syncytium, now merely moulded into the interspaces of the nerve-elements and blood-vessels, begins to show appearances indicative of the adult form. Nuclei situated in the larger interspaces have pressed about them a more compact protoplasm which shows slight granulation (endoplasm). These masses are continuous with those in neighboring spaces by necessarily more or less attenuated portions of the syncytium (exoplasm), which appear more fibrillated than the portions immediately about the nuclei (see *a*, Figs. 12 and 13). Whether in large or small masses, the more fibrillated portion of the syncytium stains less deeply than the more granular areas. Its occurrence is probably the first evidence of the transformation of the tissue, for it is often apparent that the more deeply staining form, usually about the nuclei, is being converted into the less deeply staining form. When the "free nuclei" occur in spaces large enough to afford an appreciable amount of the more deeply staining protoplasm about them, it may be assumed conversion has occurred (*d*, Fig. 12).

The more lightly staining portion of the syncytium may be considered prefibrous tissue, for it is this which becomes transformed into the neuroglia fibers. If the special neuroglia stain can be trusted to express the process, the transformation is interesting. First, more evident fibers appear in the section, seemingly formed by a condensation of the less deeply staining substance. These fibers are of various lengths and usually their course is more or less straight. They may pass through the domain of more than one nucleus. Instead of staining the characteristic blue of neuroglia fibers, most of them stain only a more dense shade of the color of the general substance. However, in the same section some also may be seen undergoing the chemical transformation (*f*, Fig. 12). In such a fiber a portion only may give the blue reaction, while another portion may stain indistinctly blue or not at all. Close examination sometimes reveals something like a line of fine dots (*e*, Fig. 12). These fibers vary but little in size and are but little smaller than those found in the adult.

It is true that in the adult also, where blue-staining fibers are abundant, both unstained fibers and fibers showing some of the above features may be observed. Whether such appearances in the adult indicate imperfect staining or the development of new fibers, is difficult to say. Probably they indicate both. But when found in a stage before which no fibers have taken the neuroglia stain, the question is somewhat different. There are evidences that the process of both development of fibers and transformation does continue into the adult stages.

In the field chosen in Fig. 13 (suckling pig of two weeks) the conditions resemble those in the adult. Throughout the section, however, neuroglia fibers are not so abundant as they are in the adult. On the other hand, fibers in the process of transformation may more often be seen and nuclei surrounded by the more deeply staining protoplasm, are considerably more numerous than in the adult. Whether in the young or the adult, a fully transformed (staining) neuroglia fiber usually appears in a clearer space, as though the surrounding substance had been used up in its development.

The field represented in Fig. 13 was chosen chiefly because of the types of nuclei contained in it. Medullation is far advanced. Either due to an increase in pressure upon the substance in the interspaces consequent to the enlargement of the axones by medullation or due to some chemical difference, or fault in technique, the masses of more deeply staining protoplasm, when present, color somewhat darker than in Fig. 12. Nuclei present in these masses give them the semblance generally described as neuroglia cells. When an interaxone space is large enough, it may contain two or more nuclei. The mass indicated by *a* in Fig. 13 both illustrates and explains the "multinucleated neuroglia cells" described in the literature (Krause, Brodmann, Aguerre). Were the axone in the center of this mass removed, the type would be perfect. As it is, it gives the appearance of three closely joined cells. It must be remembered that most of the studies of neuroglia cells have dealt with the tissue as found in the white substance rather than in the gray, where the different arrangement and less abundance of axones render the usually described form difficult to find.

Nuclei surrounded by the more deeply staining form of the syncytial protoplasm are always of the large vesicular variety. Both because of this and because of the fact that for quite a period of the embryonic development all the nuclei are of this type, the large vesicular nuclei are considered the more primary or least modified form. There may be seen nuclei with the more deeply staining substance partially converted (*c*, Fig. 13) and free nuclei (*d*). Free nuclei may be of the vesicular

variety. In fact, they probably do not begin to change till after they are free. I can only explain the remaining types of neuroglia nuclei as resulting from the shrinkage and possibly a deterioration of the vesicular type. First, there is a decrease in size (*g*, Fig. 13), resulting further in a condensation of the chromatin (*h*), and finally a much smaller, usually spherical, deeply staining form (*k*).

In the two adult hog specimens from which I made preparations, the small deeply staining forms of nuclei were somewhat more abundant than in the adult human. This may have been due to faulty technique. In the adult especially a nucleus of the smallest type may be occasionally seen which is apparently undergoing fragmentation. This phenomenon was described in the neuroglia of the elephant and further study of the nuclei in the hog tends to strengthen the assumption that most of the types are transition forms of the large vesicular variety; that having to do with the growth and transformation of the syncytial protoplasm, certain of the nuclei run a slowly terminating course, and finally suffer gradual karyolysis, while others may maintain to take part in further growth of the neuroglia. In the adult, vesicular nuclei are present with the more deeply staining protoplasm about them, as well as without it.

Of the various classifications of neuroglia nuclei in the literature, Weigert's is, I think, more nearly correct. He describes them in the human as (a) large vesicular; (b) small, deeply staining, and (c) transition forms between the two. Much more complicated classifications have been made (Aguerre, oo, and others).

As to *the nature of the neuroglia fibers,* the conclusions originally subgested by Weigert are undoubtedly correct. The fibers cannot be regarded in any sense as processes or outgrowths of the cells, for they are both morphologically and chemically different from the cell-protoplasm. Furthermore, it may be added that, unlike cell-outgrowths, they often pursue an unbroken course, not only through the entire domain of the cell, but through the domain of several cells. In other words, they are fibers distinct from the protoplasm but derived from it. They are small, vary but little in thickness, and are of indefinite length. Their chemical differentiation is seemingly the last stage of their development. Unless the entire syncytium be considered as a cell (a nucleated mass of protoplasm), the fibers are intra-syncytial in origin rather than either intra- or extra-cellular.

After medullation, and in the adult of all the forms I have examined, there remains a peripheral excrescence, or cortical layer, of the syncytium, which is unoccupied by the neuraxes of the spinal cord. In the elephant (referred to as the "marginal veil") it is considerably thicker

than in the human or hog. Being void of axones, of course no "neuroglia cells" appear in it. Its nuclei are simply embedded in a formless, fibrillated protoplasm. By the silver method, being on the periphery of the spinal cord, it is almost invariably clogged by the deposit, and being thin is usually passed unnoticed. By the differential neuroglia stain it appears as a dense plexus of blue-staining neuroglia fibers and shows in sharp contrast with the adjacent pia mater, which is colored a light, reddish-brown. The plexus of neuroglia sends processes among the axones along its inner border. These processes simply express its continuity with the general syncytium of the entire specimen.

Some of the steps in the processes of the development and transformation of the neuroglia fibers are, as I see them in the pig, similar to certain of the processes described by Mall, 02, in the development of the white fibrous tissue from the connective-tissue syncytium. Since neuroglia, the chief fibrous-supporting tissue of the central nervous system, bears a close resemblance to white fibrous tissue in certain of its framework arrangements, it would not be surprising if a close comparison of the two should reveal similar stages in their development.

A summary of the entire processes of the development and transformation of the neuroglia is given in the last section of this paper.

Experiments with Digestion.

The results of the application of the digestion method to the embryonic and adult spinal cord have been disappointing as to their contributing to a knowledge of the growth and properties of the neuroglia. In the early stages, before medullation and before neuroglia fibers make their appearance, the results obtained by digestion are quite positive. Up to 6 centimeters the embryonic spinal cord is so friable that it was found necessary to remove the segments intact in the vertebral canal and subject the whole to digestion. In this way one could be sure the piece of spinal cord was not lost in the manipulation.

The syncytium of the embryonic spinal cord digests in common with the connective-tissue syncytium. In all the first stages the spinal cord digests out entirely, and, also, even at 3 centimeters, there is left scarcely a vestige of the embryonic meninges. In pigs of 4 centimeters, however, while the spinal cord digests totally, the anlage of the dura and pia mater, though thin, begins to positively resist digestion. At this stage the meninges begin to stain by Mallory's method for white fibrous tissue. Thus it is seen that as stainable and indigestible membranes (white fibrous tissue), the meninges are evident long before neuroglia fibers make their appearance.

Up to 16 centimeters the spinal cord digests out, leaving only a thin cuff, the pia mater, with a fringe of delicate processes projecting from its inner surface. These processes represent the walls of blood-vessels and the ingrowths of the pia into the specimen.

In pigs of 20 centimeters, digestion leaves results but slightly different from the 16-centimeter stage. Though, as previously found, medullation is well underway at 20 centimeters and neuroglia fibers are beginning to appear, it seems that there is not yet developed in the spinal cord a framework sufficiently resistant to maintain even a delicate phantom of the section. If the neuroglia fibers resist digestion, they must be so few and dissociated that they are washed out in the process. The cuff of pia is somewhat thicker than at 16 centimeters and its processes into the cord are somewhat thicker and longer, but apparently none of the framework of the developing medullary sheaths is maintained.

At 28 centimeters the white substance has attained an evident resisting framework, while the entire gray figure digests out practically clean. The resulting opening in the transverse sections, giving a good outline of the gray figure, is lined with a delicate fray of ingrowths. A narrow space appears between the white substance and the pia mater, making it seem as though the two are detached except for the blood-vessels and pial ingrowths.

The spinal cord of the suckling pig and of the adult behave much alike when subjected to digestion. In the young relatively more substance is removed from the gray figure than in the adult, and in the latter, of course, all resistant structures are thicker and more closely associated than in the young pig.

In the adult spinal cord, both of the hog and human, a marked framework resists digestion just as the connective-tissue framework of other organs of the body does. It is well known that developed white fibrous tissue resists the action of the pancreatin and, as first shown by Ewald and Kühne, **76**, and by Rumpf, **78**, the framework of the medullary sheath resists digestion much as white fibrous tissue does.

The intermixing of the white fibrous components of the spinal cord with the neuroglia renders it difficult to determine the behavior of the neuroglia in digestion. It can be said with certainty that all the protoplasm in the untransformed state, whether of ectodermal or mesodermal origin, is removed by the action of the ferments, but it is not certain with reference to the neuroglia fibers. The difficulty lies in the nature of the digested preparations, and chiefly, perhaps, in the question of staining. I have been unable to devise an application of the special neuroglia stain which will differentiate neuroglia fibers in sections of the

digested material. All the resisting tissues stain practically the same. Either the neuroglia fibers are digested out or, in the process of digestion, the very properties upon which their differential staining is based are destroyed. Undifferentiated, the presence or absence of neuroglia fibers in the section is hard to determine, because, with the best of care, the various washings necessary in staining the sections of digested material result in a further collapse of the finer structures and a coherence or washing aside of the individual fibers. Also, while at times individual fibers may be seen which resemble neuroglia fibers, being undifferentiated, it is uncertain whether they are neuroglia or fibrils of white fibrous tissue. Digested preparations are of such a nature that, though they may look promising under low power, they are very disappointing when examined with the oil immersion, and this is necessary in the study of neuroglia. Nothing in my preparations positively suggests the digestion of neuroglia. The partial detachment of the pia from the white substance seemed at first sight to indicate that the cortex or marginal veil had dissolved out, but under high power the general collapsed condition of the resisting structures at the periphery is such that a definite statement to this effect is impossible. The separation of the pia from the cord may be due entirely to the general swelling and loosening of the pia produced in the digesting process. Of the section in general, however, one can but say that if all the resisting framework of the spinal cord is developed from the ingrowths of mesodermal tissue solely, then mesodermal tissue must contribute for its support to a hitherto unaccredited extent.

A study of the resistance to digestion of the framework of the medullary sheath is of interest, but since these structures probably have nothing whatever to do with the neuroglia, their behavior is of necessity omitted in this paper.

The Occurrence of Nerve Corpuscles, or Seal-ring Cells, in the Central Nervous System.

There is one feature, however, to be observed in my preparations of the developing spinal cord to which I wish to call brief attention, though it also may not be concerned with the neuroglia. It is the occurrence of cells encircling the medullating neuraxes of the central nervous system. At present only a mere mention of these structures is possible. An attempt to give them a more detailed study will be made at another time.

These cells appear clasping the growing medullary sheath and resemble a seal ring in shape, with their one nucleus in the thicker side (*s*,

Figs. 12 and 13). These *seal-ring cells* have definite boundaries and therefore cannot be confused with the neuroglia nuclei surrounded by a mass of the syncytial protoplasm. They usually encircle the medullating axone completely, though sometimes their protoplasm is so thin on the side opposite the nucleus that they appear as a crescent rather than a circle. They are not noticed till medullation has begun and they appear more frequently about small and medium-sized azones than about those whose medullary sheath has attained the larger proportions. They seemingly increase in size with the thickening of the medullary sheath they encircle. That shown in figure 13 is the largest I have observed in any of my sections. They are more numerous during the period of most active medullation (16 to 25 centimeters) and, so far, I have been unable to note distinct examples of them in the adult material. If they occur in the adult at all, their protoplasm must be either exceedingly thin, or all used up, and their nuclei may be included among the apparently "free neuroglia nuclei," some of which are often seen lying close or even curved upon the periphery of the medullary sheaths. I have never seen more than four of these cells in a single field of the oil immersion. More often a field contains none at all. The cell shown in Fig. 13 (*s*) is entirely unique in the preparations. It apparently involves two axones in its clasp. Whether this is due to pressure or is indicative of the nature of these cells, it is the only one observed behaving in this way.

As to the function of these seal-ring cells, it is at present assumed that they bear the same relation to the medullated axones of the central nervous system as that ascribed to similar cells known to occur in the peripheral nervous system. Adamkiewicz, **85,** was, I think, the first to fully describe such cells in the developing peripheral nerves. He referred to them as "nerve corpuscles" and "half-moon cells," and they have since been called "Schwann's corpuscles" from their relation to the sheath of Schwann and their supposed identity with the nucleus of that sheath in the adult peripheral nerve. These cells are thought to be actively concerned in the development of the myelin sheath. Considering the seal-ring cells in the developing central system as similar to the nerve corpuscles in the peripheral nerves, it may be assumed that in the central system also they have something to do with the development of the medullary sheaths and, further, that they are likewise of mesodermal origin, probably recruited from the wandering cells which are known to enter the central system. Whether or not in either system these cells play a rôle in the production of myelin similar to that played by the fat-cell in the production of fat, it may at least be advanced that they

have to do with the supporting structures of the myelin sheath, for this sheath in the central as well as in the peripheral system possesses a primitive sheath and a delicate framework throughout, contributing to its organization. In the central system the primitive sheath is much thinner than in the peripheral nerves, but it may be discerned in preparations from which the myelin has been extracted, and also the delicate framework which permeates the myelin itself. Some of the features of this framework have been recently described by Wynn, 00, and Hatai, 03, who give the findings of previous observers, and my preparations show that both the primitive sheath and the framework not only resist digestion, as first shown by Ewald and Kühne, 76, but also, after the special neuroglia method, they both stain like the mesodermal tissue from the pia mater.

SUMMARY.

1. The cells composing the neural tube are at first individual and definitely arranged, but at an early stage they all lose their boundaries and the resulting fusion of their protoplasm gives rise to a syncytium.

2. The protoplasm of the syncytium increases more rapidly than the nuclei are distributed and, in consequence, there appears at the periphery of the embryonic spinal cord an excrescence of non-nucleated protoplasm, which becomes the mantle layer of the later stages.

3. The fine threads of the spongioplasmic network of the original cell-protoplasm thicken and the meshes enlarge, giving rise to a filamentous reticulum, and, at the peripheral and ventricular surfaces of the neural tube, this reticulum becomes condensed into the external and internal limiting membranes. Thus the specimen becomes a reticulated syncytium with definite boundaries.

4. The threads further thicken both by growth of their substance and by a condensation of adjacent threads, resulting from the collapse of many of the smaller meshes occasioned by a radial drawing out of the reticulum. This radial drawing out and condensation is due partly to the original form of the syncytium, but largely to the lateral direction of the growth of the wall of the tube and the radial migration of the nuclei from the ependymal layer toward the periphery. It continues till the syncytium of the lateral wall assumes the form of radially arranged, axial filaments connected with each other by numerous smaller threads between them. Due to the nature of their formation, the axial filaments apparently bifurcate near the inner boundary of the mantle layer. Here the bifurcations, together with the more numerous lateral threads, result in a complexity in the arrangement of the filaments which, for a time, prevents the nuclei from migrating into the mantle layer.

5. The ingrowth of blood-vessels into the neural tube first occurs in pigs of from 9 to 10 millimeters.

6. Prior to the ingrowth of blood-vessels, all nuclear division occurs in the ventricular border of the ependymal layer and, after the ingrowth of blood-vessels the great majority of the dividing nuclei and most of those of undoubted ectodermal origin occur in the ependymal layer. Mitosis increases from the earliest stages, reaches and maintains for a time a period of maximum activity (pigs of 10 to 20 millimeters), then gradually declines and, in pigs of from 30 to 40 millimeters, practically ceases altogether.

7. Throughout the lateral walls of the neural tube, the nuclei migrate radially from the ependymal layer, first from the ventral half of the layer, forming the anlage of the ventral horn, and then a general migration giving rise to a middle layer of the specimen with nuclei more loosely arranged then in the ependymal layer. The mantle layer remains practically uninvaded by ectodermal nuclei till in pigs of 70 to 80 millimeters.

8. The ventricle of the neural tube increases in size in pigs up to about 30 millimeters, then it decreases by a collapse of its dorsal two-thirds, and there results a central canal but little larger than that of the adult. Coincident with the collapse of the ventricle, nuclear division ceases and the continued migration of the nuclei results in a thinning of the ependymal layer, hitherto maintained quite thick, till the layer becomes an ependyma similar to that of the adult.

9. With the present technique, there is nothing to show that all the products of the mitoses (germinal cells) in the ependymal layer are not indifferent elements from the first—capable of developing into either neurones or neuroglia.

10. The syncytium of the neural tube, at first wholly of ectodermal origin, soon becomes invaded by ingrowths of the connective-tissue syncytium without and by two other types of mesodermal elements. The fact that the syncytia from the two sources fuse before the neuroglia is formed as such, and the fact that the nuclei from the two sources are similar, make it difficult to determine whether mesodermal elements take part in the formation of the neuroglia or not. Tissue of mesodermal origin contributes appreciably to the supporting tissue of the spinal cord but, by definition, such cannot be called neuroglia.

11. The differentiation of the fibrils of white fibrous tissue occurs considerably before the appearance of neuroglia fibers.

12. Soon after the cessation of mitosis, the radial arrangement of the syncytium of the spinal cord becomes obliterated by the further ingrowth

and elaboration of the nerve-elements and blood-vessels, the arrangement being finally maintained only in the, then thin, ependymal layer. With the beginning of medullation (pigs of 16 to 20 centimeters) the syncytium becomes moulded in the interspaces of the neurones and blood-vessels and, for the first time, there begin to appear in the white substance the characteristic shapes hitherto described as neuroglia cells.

13. The silver method cannot be trusted to tell the true story, and never the whole story. As to the pictures of neuroglia cells obtained by this method, while most of them probably contain nuclei, many of them do not.

14. The development of neuroglia fibers from the syncytium is a process of transformation. Only fully developed (chemically transformed) fibers react to the special neuroglia stain. These do not appear in pigs till after the processes of medullation are well underway. The method of their development is somewhat similar to that described for the fibrils of white fibrous tissue.

15. In the early embryo all the nuclei are of the large vesicular type, and the various varieties of neuroglia nuclei described in the literature may be considered as transition forms of this type. Beginning with this type, many of them gradually decrease in size, change in their staining properties and, in their final stage, undergo fragmentation and disappear.

16. The syncytium of the spinal cord digests in common with the connective-tissue syncytium. Owing to the chemical effect of the digestion process upon the tissue, and the presence of white fibrous tissue in the specimen, and to the physical nature of the preparation, it is difficult to determine whether neuroglia fibers digest or not. The framework of the medullary sheaths in the central nervous system both resist digestion and stain as white fibrous tissue does.

17. About the medullating axones of the central nervous system there occur cells similar to the nerve-corpuscles described in the developing peripheral nerves.

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EXPLANATION OF FIGURES ON PLATES I-V.

All the figures are from transverse sections of the spinal cord, and with the exception of Fig. 1, which is from the rabbit, all illustrate conditions found in the pig (cervical region). The oil immersion (Zeiss) was used in all the drawings.

PLATE I.

Fig. 1. From the neural tube of an embryo rabbit shortly before the closure of the tube. Taken from His, 89. Showing a stage when the wall is composed of distinctly outlined, individual cells. $a =$ inner zone; $g =$ mitotic nucleus or germinal cell; $m =$ peripheral zone, position of the later mantle layer. $\times$ 920.

Fig. 2. Pig of *5 millimeters*, unflexed. Just after the closure of the neural tube. Segment from lateral wall of tube showing the disappearance of cell membranes and the beginning of the consequent fusion of the cell protoplasm to form a syncytium. $a =$ inner zone; $g =$ mitotic nuclei or germinal cells; $m =$ beginning of mantle layer; $mli =$ internal limiting membrane; $r =$ radial columns of protoplasm. $\times$ 920.

Fig. 3. Pig of *7 millimeters*, unflexed. Segment from ventro-lateral wall of neural tube. Showing the earliest form of the syncytium and the second

stage in the mantle layer. *ma* = inner zone or ventricular border of the ependymal layer; *g* = nuclei in mitosis or germinal cells; *mli* = membrana limitans interna; *mle* = membrana limitans externa; *r* = radial, axial filaments of the syncytial protoplasm; *p* = anlage of pia mater. × 920.

Fig. 4. Pig of *10 millimeters*, "crown-rump measurement." Segment from lateral wall of neural tube showing more pronounced radial arrangement of the syncytium, the final disappearance of all cell membranes, and a third stage in the structure of the mantle layer. *a* = inner zone or ventricular border of ependymal layer; *b* = boundary between nucleated layer and mantle layer; *g* = mitotic nucleus; *m* = mantle layer; *mli* and *mle* = internal and external limiting membranes; *r* = axial filaments; *p* = anlage of pia mater. By the author's mistake in measurement the original drawing is too much reduced in this figure. To conform to the scale of the preceding figures its long axis should be about ½ inch greater.

PLATE II.

Fig. 5. Pig of *9 millimeters*, flexure beginning. Showing the stage of the first ingrowth of blood-vessels, the abundance and locality of dividing nuclei, the migration of the nuclei, the beginning of the middle nucleated layer, and the general appearance of the syncytium at this stage. A portion of the surrounding connective-tissue syncytium is included in the drawing. The depression along the ventral aspect of the specimen is greater than is usual at this stage (see Fig. 6). *bc* = blood capillary; *cs* = connective-tissue syncytium; *d* = dorsal aspect of tube; *ep* = ependymal layer; *mn* = middle nucleated layer; *mv* = mid-ventral portion of mantle layer; *vh* = anlage of ventral horn; *sg* = spinal ganglion; other reference letters = same as in Fig. 4. × 170.

Fig. 6. Photograph of section from pig of *15 millimeters*, "crown-rump measurement." Showing the low-power appearance, relative thickness and extent of the mantle layer, the general appearance of the syncytium, the appearance of the middle nucleated layer (*mn*) resulting from the further migration of nuclei from the ependymal layer (*ep*). Fig. 7 is taken from the ventral portion of sections similar to this. *d* = dorsal portion of ependymal layer about which the mantle layer does not extend; *dh* = anlage of dorsal horn; *mv* = mid-ventral portion of mantle layer; other reference letters = same as in Fig. 4. × 90.

Fig. 7. Combination drawing from sections of pig of *15 millimeters*. The lower part of the drawing is taken from the ventral portion of the section shown in Fig. 6; the upper part is from a section of the same stage but stained by the silver method. The drawing allows a comparison of the results obtained by the two methods and shows in more detail the appearance of the syncytium in the nucleated layer (*mn*) and the mantle layer (*m*), and the greater complexity of the protoplasmic filaments along the border line between the two (*b*). *bv* = blood-vessel; *cs* = connective-tissue syncytium; *mv* = differently arranged mid-ventral portion of the mantle layer; *r* = radial filaments of syncytium as shown by the silver method; other letters same as in Fig. 4. × 320.

Fig. 8. Photograph of transverse sections from pig of *30 millimeters*. Showing further advancement of the changes begun in Fig. 6, including the

more fibrillated appearance of the syncytium, the central canal resulting from the collapse of the ventricle, and the thinning of the ependymal layer (*ep*) due to the continued migration of the nuclei and the cessation of nuclear division. In the dorsal horn (*dh*) the smaller variety of nuclei is more numerous than elsewhere. *i* = ingrowths of pia visible because of slight shrinkage of specimen; *ms* = septum formed by fusion of internal limiting membrane; *n* = first evidence of cell groups in ventral horn (*vh*); *sp* = beginning of posterior septum; other letters = same as in previous figures. × 90.

PLATE III.

FIG. 9. Combined drawings from sections of same spinal cord as shown in Fig. 8. Showing the increased filamentous appearance of the syncytium, the further modified structure of the mantle layer (*m*), now studded with embryonic neuraxes, the form of the mid-ventral portion of the mantle layer (*mv*) resulting from the forces of growth, and the ingrowths of mesodermal tissue (*i*). Comparing the appearance of the section with the pictures obtained in the same specimen by the silver method, the pia mater (*p*) is now positively indicated in the more compact arrangement about the periphery of the connective tissue syncytium (*cs*). Other letters = same as in other figures. × 320.

PLATE IV.

FIG. 10. Combined drawings of lateral segments from sections of spinal cord of a pig *55 millimeters* long. Showing the finely fibrillated syncytium with the radial arrangement maintained, the increased variation in the nuclei of *mn*, the increased complexity of the filaments at *b*, and the increased selectiveness exerted by the filaments (*r*) upon the silver compound. × 300.

FIG. 11. Combination drawing same as in Fig. 10, but from a pig of *70 millimeters*. Showing the beginning obliteration of the radial arrangement of the syncytium and the beginning selectiveness of other than ependymal nuclei for the silver. *b* = "neuroglia cells" without nuclei; *c* = cells probably containing nuclei; *e* = filaments detached from the internal limiting membrane; other letters = same as in Fig. 6. × 300.

PLATE V.

FIG. 12. Combination drawing from transverse sections of the spinal cord of pig of *20 centimeters*. Showing the condition of the syncytium, the first appearance of neuroglia fibers and the probable nature of the "neuroglia cells" of the silver method. *a* = neuroglia cell after the Benda method; *a'* = similar cell by the silver method; *b* and *b'* = non-nucleated neuroglia cells; *x* = mass of the syncytium with silver deposit; *d* = free nuclei; *e* and *f* = neuroglia fibers beginning to take the differential stain; *s* = seal-ring cells. × 700.

FIG. 13. Area from transverse section from suckling pig of *two weeks*. Showing fully developed neuroglia fibers and fibers in process of transformation, and varieties of "neuroglia cells." *a* to *k* = neuroglia nuclei in various stages; *s* = seal-ring cell. × 700.

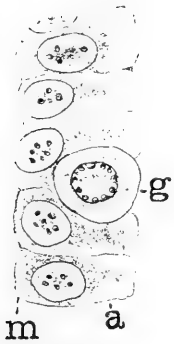

FIG. 1

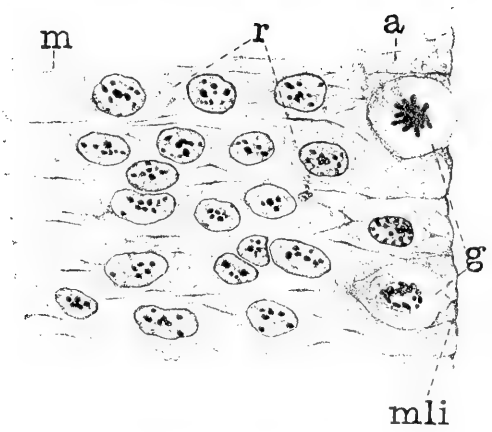

FIG. 2

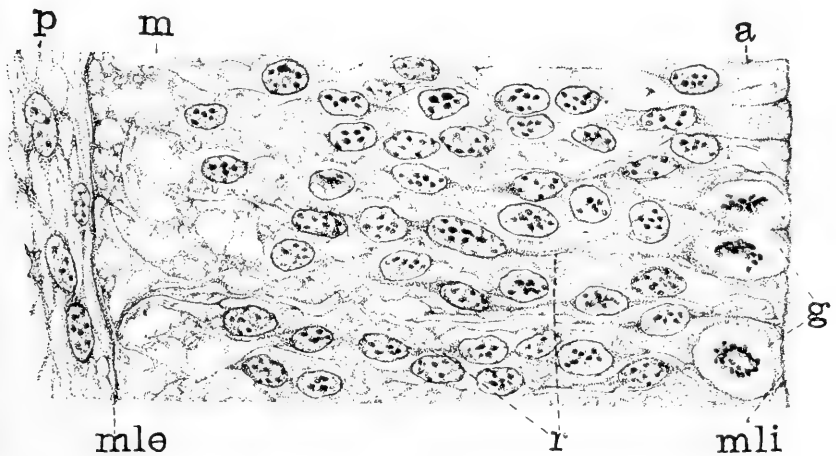

FIG. 3

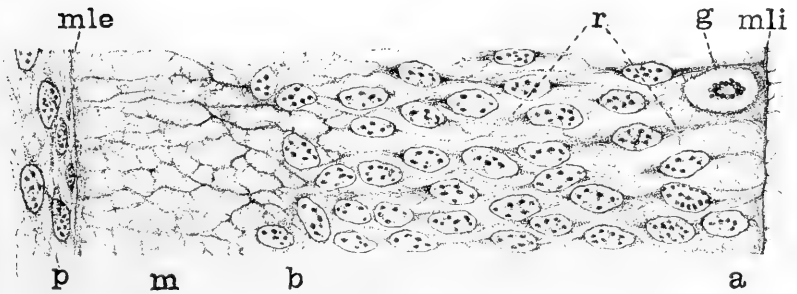

FIG. 4

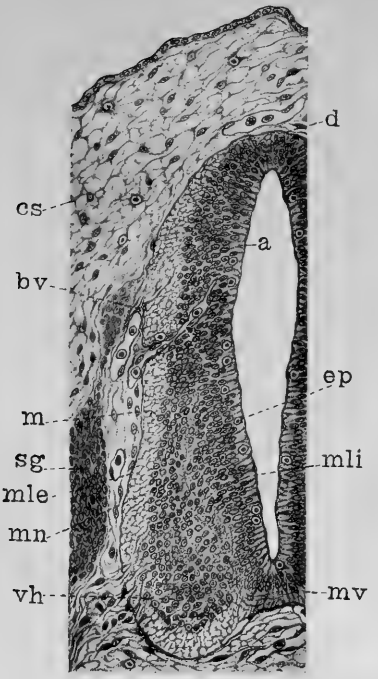

FIG. 5

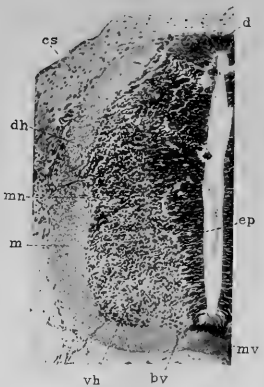

FIG. 6

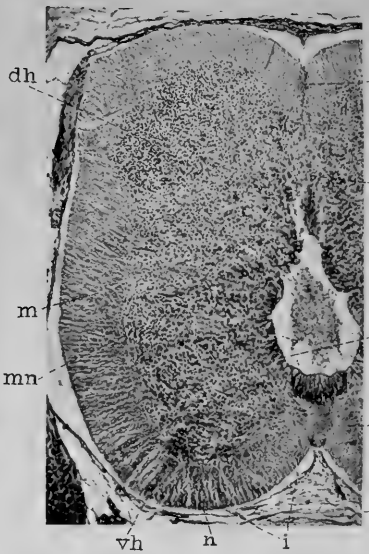

FIG. 8

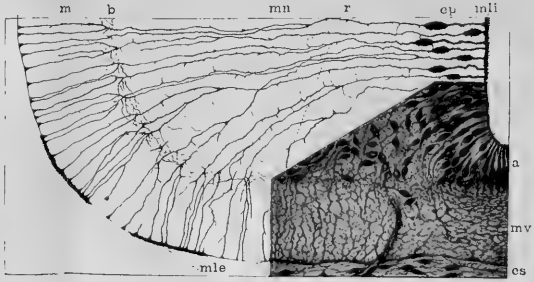

FIG. 7

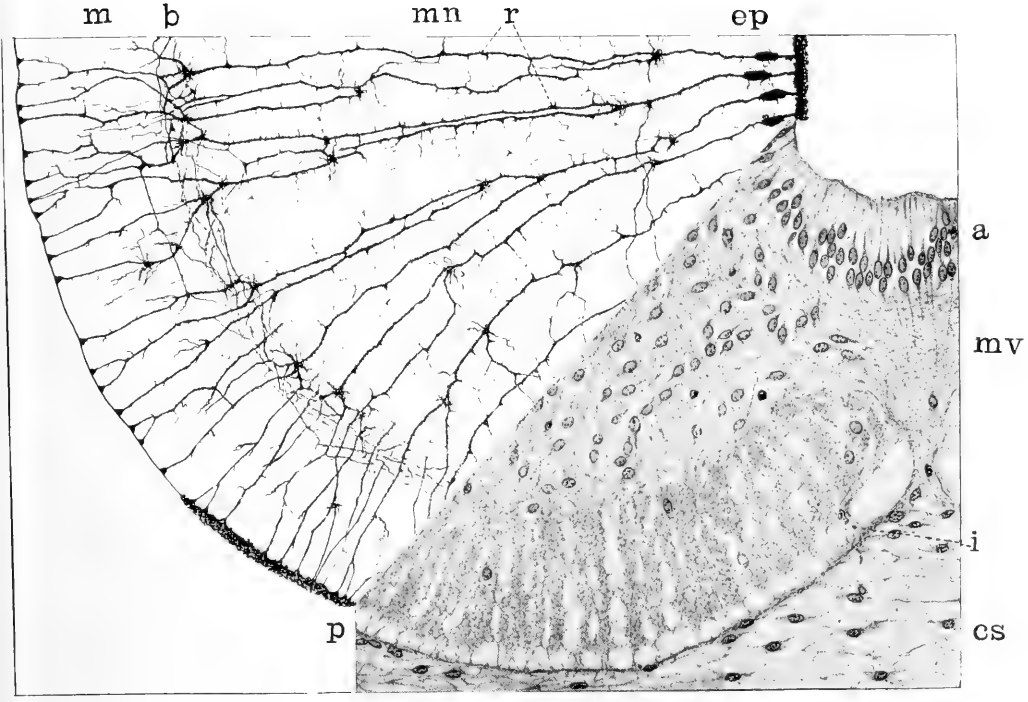

FIG. 9

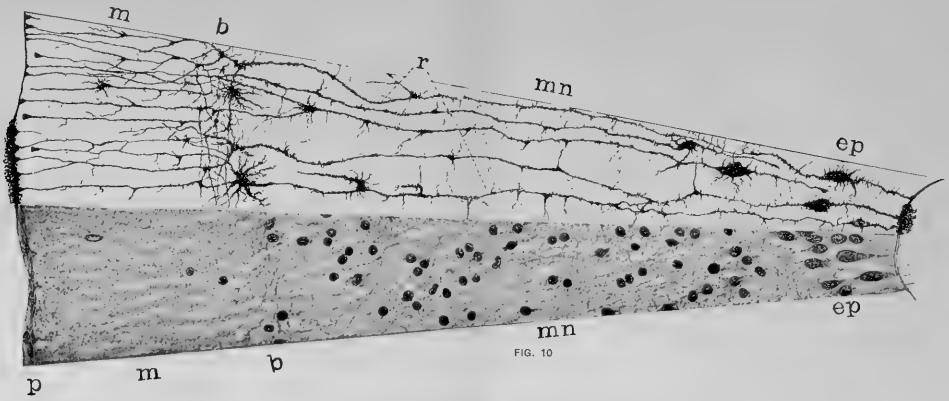

FIG. 10

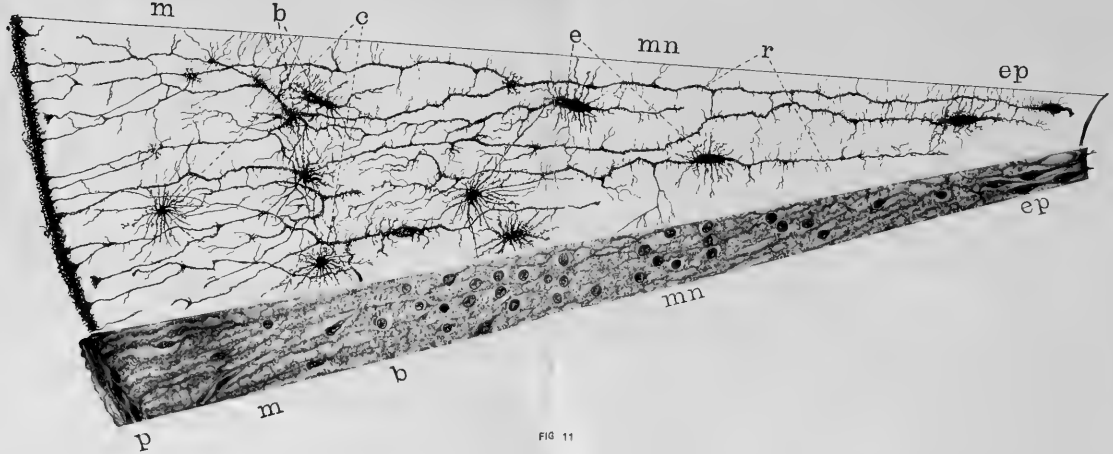

FIG 11

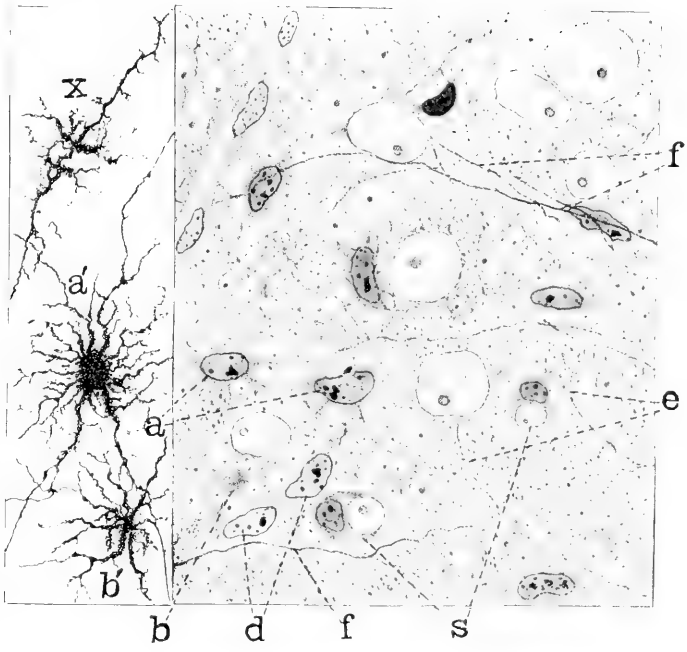

FIG 12

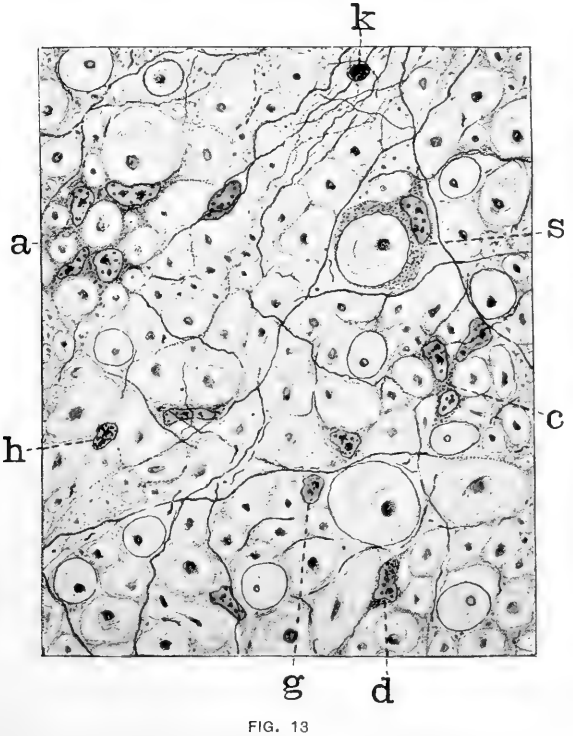

FIG. 13

THREE CASES OF A PANCREATIC BLADDER OCCURRING IN THE DOMESTIC CAT.

BY

W. S. MILLER, M. D.

From the Anatomical Laboratory of the University of Wisconsin.

WITH 3 TEXT FIGURES.

The arrangement of the pancreatic ducts in the cat is quite different from that in man or in the dog.

In man we find the ductus pancreaticus (Wirsungi) opening into the duodenum in connection with the ductus choledochus, while the ductus accessorius (Santorini) enters the duodenum nearer to the pylorus. The larger of these two ducts is the ductus pancreaticus.

In the dog the ductus pancreaticus, as in man, enters the duodenum with the ductus choledochus, but the ductus accessorius enters the duodenum caudosinstralward from the common opening of the ductus choledochus and ductus pancreaticus. The larger of these two is the ductus accessorius.

There are in the cat two pancreatic ducts, and their relation to the ductus choledochus and duodenum is practically the same as in the dog, with the exception that the ductus pancreaticus is the larger of the two ducts.

Following the nomenclature used by Owen, we find that the ductus pancreaticus is formed by the union of the two main trunks which come, the one from the splenic, the other from the duodorsal portion of the pancreas. The ductus accessorius is small, in some cases insignificant in size, and varies considerably in its mode of origin.

The ducts of the pancreas in Mammalia differ from those of the liver in that there is not usually connected with them a receptacle for the storage of the pancreatic juice; on the other hand, absence of a gall-bladder, except in the Perissodactyla, is exceptional.

In 1815 Mayer figured and described a pancreatic bladder in a cat. This bladder was situated on the inferior (caudal) surface of the liver, close to the gall-bladder, and was connected with the duct of Wirsung

by means of a rather long duct. The gall-bladder occupied its usual position and exceeded the pancreatic bladder in size.

In 1879 Gage, of Cornell, figured and described a second case of a pancreatic bladder, and, like that of Mayer, it was found in a cat. This case was not mentioned by Oppel in his excellent work on the comparative anatomy and histology of the pancreas. Gage describes his case as that of a "pancreatic reservoir, analogous to the gall-bladder. In this case it is larger than the latter and partly covers it. The two are very closely bound together for about half their longitudinal extent, by a broad, firm band, which produces a decided constriction in both. The walls of the reservoir are very firm and thick, as are also those of its ducts. The duct is nearly straight, and bifurcated before terminating, sending the larger branch to the gastro-splenic division of the duct of Wirsung, and the smaller to the common trunk. . . . There was no communication whatever between the pancreatic reservoir or its duct and the gall-bladder or the ductus choledochus."

These two cases are the only authentic ones that I have been able to find in which a true pancreatic bladder has been present. It has been reported as being present in other mammals, but I fail to find substantial proof. The dilatation of the ampulla of Vater, which is often found, as for example in the elephant and rhinoceros, situated as it is within the walls of the duodenum, is quite another thing and is not to be considered a bladder.

Three cases in which a pancreatic bladder was present have come under my observation, and like the case of Mayer and of Gage, they were found in the domestic cat. Of these three cases of pancreatic bladder, cases II and III were practically identical, while case I presented quite a different type.

In case I (Fig. 1) the pancreatic bladder occupied the usual position of the gall-bladder. The gall-bladder was about one-third the size of the pancreatic bladder and was situated to the right of the pancreatic bladder, with which it was connected by a small amount of loose connective tissue. The duct leading from the pancreatic bladder crossed the ductus cysticus just as it left the gall-bladder, passed obliquely over the right branch of the ductus hepaticus, then ran parallel to the ductus choledochus, and finally joined the duodorsal division of the ductus pancreaticus 6 mm. from its union with the splenic division. Two small ducts arising in the duodorsal portion of the pancreas joined the duct coming from the pancreatic bladder just before its union with the main duct to form the ductus pancreaticus. The lobation of the liver presented nothing abnormal; both the liver and pancreas were of normal size.

In case II (Fig. 2) the gall-bladder occupied its usual position. The pancreatic bladder was about two-thirds the size of the gall-bladder. It occupied a position caudal to the gall-bladder, with which it was loosely united by connective tissue. The duct coming from the pancreatic bladder passed obliquely over the ductus cysticus and the right branch of the ductus hepaticus, and, after running parallel to the ductus choledochus, joined the duodorsal division of the ductus pancreaticus 11.5 mm. from its union with the splenic division. In size the liver and pancreas were normal. The lobation of the liver was normal, except that the left lateral lobe had a deep incision extending from the porta hepatis transversely across it, nearly subdividing it into two portions.

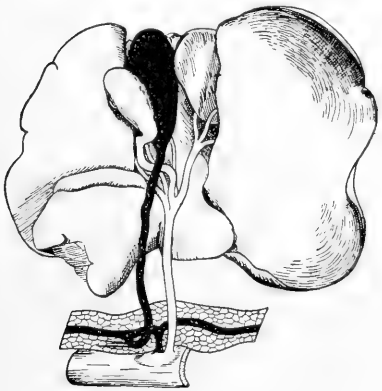

FIG. 1.

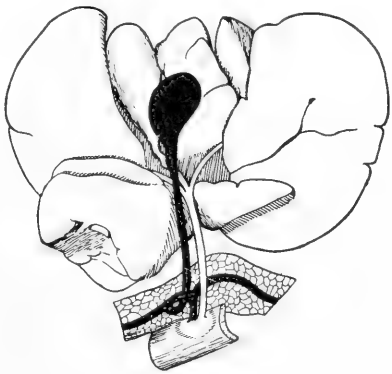

FIG. 2.

FIG. 1. Diagrammatic outline of the liver and a portion of the pancreas and duodenum in Case I. The liver has been turned cephalad. The pancreatic ducts and bladder are represented in solid black; the gall-bladder and bile ducts in outline. Note that the pancreatic bladder occupies the usual position of the gall-bladder, while the latter lies caudal to it resting on the right median lobe of the liver. The two small ducts mentioned in the text can be seen below the point of union between the pancreatic cystic duct and the duodorsal division of the ductus pancreaticus.

FIG. 2. Diagrammatic outline of the liver and a portion of the pancreas and duodenum in Case II. The liver has been turned cephalad. Pancreatic bladder and ducts black; gall-bladder and ducts in outline. The gall-bladder in this case occupies its normal position.

In case III the gall-bladder was the larger of the two and occupied its normal position. The pancreatic bladder was half the size of the gall-bladder and firmly attached to it by a strong sheet of connective tissue. The course of the duct was as in case II. It joined the duodorsal division of the ductus pancreaticus 7 mm. from its union with the splenic division. Just before its union with the main duct of the pancreas a few small ducts coming from the adjacent part of the pancreas joined it. The left lateral lobe of the liver was somewhat folded

upon itself, otherwise the liver showed nothing unusual; the pancreas was normal.

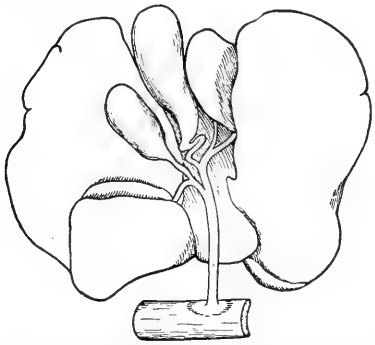

Fig. 3. Diagrammatic outline of the liver mentioned in the text in which two gall-bladders and cystic ducts were present.

It may be of interest to note that two of the above-described cases came from the same farm house and that the third came from a neighboring house. In a full brother of case II, two gall-bladders were found. There was a well-developed gall-bladder and cystic duct connected with each branch of the ductus hepaticus (Fig. 3). Of these two gall-bladders the one connected with the left branch of the ductus hepaticus was the larger and occupied the usual position of the gall-bladder, while the one connected with the right branch was about half the size of the other and occupied a special depression on the ventrocaudal surface of the right median lobe.

The year following that in which the above-described pancreatic bladders were found, two animals were obtained from the same neighborbood, and they presented the following variations in the pancreas. In one case there was a long narrow band of pancreatic tissue extending along the ductus choledochus nearly as far as the gall-bladder; its duct joined the duodorsal division of the ductus pancreaticus. In the other case there was a duct arising from the duodorsal division of the ductus pancreaticus which ran parallel to the ductus choledochus, and in place of terminating in a bladder was connected with a small truncated mass of pancreatic tissue situated in the fossa vesicæ felleæ. May not these two cases explain partially the way in which the pancreatic bladders have been formed?

All three cases of pancreatic bladder differed from those of Mayer and of Gage in that the duct coming from the pancreatic bladder joined the duodorsal division of the ductus pancreaticus.

In one case the pancreatic bladder was the larger and occupied the usual position of the gall-bladder; in the other two cases the gall-bladder was the larger and occupied its normal place.

In two cases one or more small ducts joined the duct coming from the pancreatic bladder just before it united with the duodorsal division of the ductus pancreaticus. In the third case no such branch was present.

MAYER, A. C.—Blase für den Saft des Pancreas. Deutsches Archiv f. Physiol., Bd. I, 1815.

GAGE, S. H.—The Ampulla of Vater and the Pancreatic Ducts in the Domestic Cat. Amer. Quart. Mic. Journal, Vol. I.

OPPEL, A.—Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbelthiere. Bd. III, Jena, 1900.

ON REGENERATION IN THE PIGMENTED SKIN OF THE FROG, AND ON THE CHARACTER OF THE CHROMATOPHORES.

BY

LEO LOEB AND R. M. STRONG.

From the J. R. H. Molson Laboratories of Pathology and Bacteriology, McGill University, Montreal.

In a former paper L. Loeb, **97**, described the changes occurring in the pigmentation of regenerating black skin on the ear of the guinea-pig, and after transplantation of pigmented skin into white skin. In this paper we give the results of a study of the conditions in regenerating frog skin. We shall consider principally the chromatophores and their origin in the epidermis, and add some notes on histological changes involved in the regeneration. We also give a brief statement of the results of some experiments with atropine and pilocarpine solutions.

These later experiments were undertaken with the intention of investigating the influence of different substances upon the growth of tissues in higher animals. In earlier experiments one of us (Loeb) had investigated the influence of narcotic substances like alcohol and chloroform upon the regeneration of the tail in tadpoles. In these experiments atropine and pilocarpine were chosen, because Matthews, **02**, had more recently found that pilocarpine accelerates the development of fertilized ova of Asterias somewhat.

Methods and Material.—A patch of skin, 5—8 mm. long and 3—5 mm. broad, usually elliptical, but sometimes oval in outline, was removed from a black area on the dorsal surface of the left shank of each frog. The animals were in apparently good condition and mostly very active. Before operation they were kept at the laboratory for several days in large battery jars containing water about 1-1½ inches deep.

After the removal of the patch of skin, the frogs were divided into three series and placed in jars. The animals of one series were in jars containing tap water; another series was given a solution of atropine sulphate and a third lot of frogs had a solution of pilocarpine hydrochlorate. Both solutions had one part of the salt to 10,000 parts of water. A few animals also were placed in solutions of 1-1000 strength.

The solution and the tap water for all these series were changed every day for the first week and every other day thereafter. At the end of periods varying from ten hours to five weeks, frogs were killed and the tissue about the wounds removed.

Corrosive-acetic was used for fixing and the material was embedded in celloidin. Serial sections were made and stained in Delafield's hæmatoxylin and eosin. Altogether, series of sections from 62 frogs were examined.[1]

Observations.—No constant effects on regeneration have been noticed for the atropine and pilocarpine solutions used. Regeneration of both epithelium and connective tissue seemed to take place equally well in either the weaker atropine, the pilocarpine solutions, or the tap water. There was no marked difference in the number of mitoses when these solutions were used. In some cases there was a little evidence of possibly greater activity in regeneration, for some cases, in the pilocarpine solution than in the atropine. This difference was very slight and possibly accidental. In one series of experiments the number of leucocytes immigrating into the wound was decidedly larger in the pilocarpine solutions. This difference, however, was not observed in a second series. Frogs kept in either the weaker or the stronger solutions of pilocarpine did not behave differently from those in tap water, even at the end of four or five weeks; they were nearly all equally active. The animals placed in the stronger atropine solution were very stupid and helpless at the end of the first day or two, apparently being partially paralyzed, and none lived longer than five days. The weaker solution seemed to have a somewhat similar but much milder effect in a few cases.

In the former series of experiments carried out in the spring of 1901, and referred to above, tadpoles, whose tails had been cut, were kept in 1-6 per cent solutions of alcohol and in weak chloroform water up to six days after the operation; the control animals lived in tap water. These experiments were undertaken in order to ascertain whether or not these narcotic substances delay and weaken the movements and the growth of the cells. No marked differences in regeneration were observed between the different sets of tadpoles, with the possible exception of slight differences in the rapidity with which the epithelium covered the wound.

Regeneration of the Epidermis.—Barfurth, **91,** observed in the Salamander a rapid movement of epithelium over a wound before any in-

[1] **A part of these investigations was carried on with the aid of a grant from the Research Fellowship Fund of McGill University.**

crease in the number of mitoses could be seen. We have made the same observation in the case of wounds in frog skin. The movement of epithelium is not limited to one layer of cells but involves several. The lower portions of the cells in the deepest layer of the epidermis move first toward the wound, and the cell-axes are occasionally rotated almost as much as 90° in the process, so that they come to have a horizontal position in place of the former vertical direction. This movement of the epithelium begins during the first hours after the operation and is completed within one to three days. There is no distinct increase in the number of mitoses, however, until after the second day.

The rapidity with which the epithelium moves over the wound after operation, together with the absence of any increase in the number of cell divisions normally occurring, indicates that the movement is not due to cell-proliferations[2] but to other causes.

Some observations made by L. Loeb on regenerating tadpole epithelium seem to indicate considerable tension in the regenerating epithelium which may be more or less responsible for the movements. He found papillæ which had ben formed apparently through the folding of the upper layers of regenerating epidermis, though they may have been the result in some cases of a degeneration of epithelial cells. These papillæ may occur within a few hours after the operation or a few days later.

The changes determining the movements affect the cells nearest the wound first, but are later extended to the epithelium farther away. The epithelium moves only in contact with solid bodies.

Mitoses occur not only in the deepest layers of the epidermis but also as high as the fifth or sixth layers, whereas in regenerating guinea-pig's skin, they were found almost exclusively in the two lowest layers.

Within forty-eight hours after the wound is made hypertrophy is seen. Individual cells enlarge and become more and more numerous.

We almost invariably find degeneration of epidermal cells connected with this hypertrophy. Such degeneration is also seen in the tissue which has advanced farthest over the wound, and is also found in the deepest layer of the epithelium in cases where many leucocytes penetrate this tissue. The nucleus of degenerating cells usually becomes kayorrhectic and the cytoplasm homogeneous, staining well with eosin. The degenerative changes accompanying the hypertrophy may be found even

[2] The proliferation of cells in the regenerating epithelium takes place both by mitosis and amitosis. Amitosis was first described for regenerating mammalian epithelium by L. Loeb, 98; later by Marchand, 01; and Werner, 02; and Nussbaum, 82, has observed amitosis in regenerating epithelium in the cornea of amphibia.

in cases where the connective tissue underlying the hypertrophied epithelium has regenerated perfectly. These degenerations are accompanied by cell-inclusions, which are sometimes almost indistinguishable from red blood corpuscles. It was found by L. Loeb, **02,** that epithelial cells in regenerating mammalian skin do actually take up blood corpuscles and other solid particles.

In one case, 14 days after the operation, a development of epithelial pearls had taken place in the regenerating and hypertrophied epithelium of a frog which had been kept in a solution of atropine. Epithelial pearls could also occasionally be seen in the guinea-pig epithelium which was growing in agar. We believe that these morphological changes do not indicate a tendency of this epithelium to assume a carcinomatous character, an interpretation which has been given to similar formations by certain investigators.

It was not uncommon to find processes of the regenerating epithelium penetrating the coagulum beneath the wound. They may advance in different directions, either in one layer or in several layers of cells. Often the fibrin fibers are merely bent inwards by the advancing epithelial cells, but they are sometimes actually perforated by the epithelium.

This penetration of the fibrin may occur within twenty-four hours after the operation, and processes in the epithelium may be observed in the fibrin as late as ten days. The cells in these processes multiply mitotically, and mitosis occurs in the epithelium lying directly on the coagulum, also, just as was the case in the experiments of Loeb for epithelium penerating coagulated blood-serum and agar.

Regeneration of the Cutis.—Though regeneration in the epidermis begins within a few hours after the operation, it does not appear in the cutis until the fifth day. When once started, however, the regeneration is frequently rapid. After six days, or a day or so from the beginning of regeneration in the cutis, a small defect may be entirely filled by connective tissue and capillaries; at later periods it was sometimes impossible to detect the wound. The position of the former wound was recognized in one case at the end of three weeks only through the presence of small mononuclear cells (lymphocytes?) in the connective tissue. In another case, taken thirty-four days after the operation, masses of small round cells in the cutis indicated the previous existence of a wound; the connective tissue had not regained its typical structure.

In a number of cases where the defect in the cutis was comparatively large there was either no regeneration of connective tissue or it was more or less incomplete. Though, in many cases, a variable number of leucocytes were frequently present in the fibrin, this was not always the case,

and it seems unlikely that such failures in regeneration were due entirely to infection by micro-organisms. Those connective-tissue cells that advance into the fibrin quite frequently degenerate; they swell up and their nuclei are destroyed by chromatolysis.

In the case where the cutis did not heal, masses of leucocytes were found in the epidermis at some places. It has not been possible to decide whether this condition was due to an invasion of leucocytes into the epithelium after which a destruction of epithelial cells followed, or whether on the contrary a degeneration of the epithelial covering, caused by imperfect healing of the connective tissue, was the primary factor, resulting, secondarily, in an immigrating of leucocytes.

A number of small gland tubules were seen under the regenerating epidermis in three wounds, at the end of the third week in two cases, and, after 34 days, the third one had gland cells dividing mitotically. In these cases only the most superficial portion of the cutis had been removed with the epidermis. In the skin adjoining the wound, typical large glands were present. It seems likely that we have here a regeneration of glands, but, as in these cases only a small part of the cutis had been removed with the epidermis, it has been impossible to determine whether or not such glandular regeneration starts from gland cells left in that part of the cutis not removed, or in the epidermis itself.

The Chromatophores of the Regenerated Tissue.—There has been no unanimity of opinion concerning the origin of chromatophores, or pigment-bearing cells with ramifying processes, in the epidermis. The earlier views, that they are immigrated leucocytes, or common connective-tissue cells that have invaded the epidermis, have been more or less generally abandoned. At present two views are held, either (1) that all chromatophores of the body are of common mesodermic origin, or (2) that the chromatophores of the epidermis are simply modified epithelial cells. This latter view has been held by a number of writers, including Kodis, Jarisch, Post, Kromayer, and ourselves, Loeb, **97,** Strong, **02.** According to the first view, all chromatophores, at a certain stage of embryonic development, are differentiated from ordinary connective-tissue cells, and a part of them grow secondarily into the epidermis. These are called melanoblasts by Ehrmann, **96,** the main exponent of this idea. Ribbert, **01,** holding the same opinion, believes that the pigmented tumors, arising from pigmented nævi of the skin, are composed entirely of such cells, and accordingly calls them *Melanomata* to designate their genetic difference from other tumors.

One of the aims of our studies was to compare the behavior of the chromatophores and the pigmentation of the regenerating frog skin with the pigmented skin of the guinea-pig during regeneration.

The pigmentation of frog skin differs considerably from that of the guinea-pig. In the frog cutis there is usually a well-marked layer of chromatophores, which are generally much larger and frequently more branched than the epidermal chromatophores, whereas the guinea-pig cutis has no well-developed chromatophores and its pigment is distributed irregularly in masses or clumps. The dermal chromatophores of the frog are separated from the epidermis by considerable connective tissue, and the epidermal chromatophores are usually situated higher up in the epidermis than is the case with the guinea-pig.

L. Loeb, 98, distinguished four stages in the development of pigmentation in the regenerating black skin of the guinea-pig. These were not observed in the regenerating frog skin.

As in the case of the guinea-pig, we find no evidence of an immigration of dermal chromatophores into the epidermis of the regenerating frog skin.

The epithelium, which moves over the wound soon after the operation, carries chromatophores and ordinary pigmented epithelial cells. These chromatophores are usually found to be without processes. During the first two weeks similar chromatophores are frequently observed in the regenerating epithelium. They may still be found during the third week, especially in the central part of the regenerated epithelium. Under these conditions they may appear as ordinary pigmented epithelial cells; they carry, however, more pigment than the latter. Kromayer has also observed chromatophores without processes near the margins of wounds in amphibia.

The number of well-developed chromatophores with large processes increases gradually in the regenerating epithelium, and they become especially numerous near the margins of the regenerating area. Chromatophores without processes were found, however, even at later periods, in the hypertrophied epithelium where cell-degeneration occurred.

Chromatophores divide mitotically during regeneration in frog skin. Two chromatophores were found in mitosis *at places where ordinary epithelial cells were also dividing mitotically,* one at fourteen and the other at nineteen days after the operation. One showed processes but the other had none.

In regenerating epithelium, the chromatophores are not arranged in as regular a manner as in ordinary epithelium. During the first two weeks many chromatophores of the epithelium covering the wound and occasionally also of the adjoining epithelium, are carried into the upper part of the epidermis and are frequently cast off. Sometimes the chromatophores are pushed into the lower layer of the epithelium, *and even*

farther into the underlying fibrin; they never come from the underlying tissue into the fibrin. Under these conditions they usually lose their processes. Near such places the fibrin may be entirely free from connective tissue. In some cases, taken at different times during the first three weeks, the skin adjoining the wound was unequally pigmented, and the regenerating epidermis often varied correspondingly in pigmentation. On a side where the epithelium adjoining the wound was more heavily pigmented, there were more chromatophores in the regenerating epidermis over the wound than at another place where the adjoining side was less pigmented.

Instead of the common arrangement of pigment on the outer side of the nucleus, which is characteristic of normal epithelial cells, we often find an irregular arrangement of the pigment in the cells of regenerating epithelium, which is probably due to the turning of the cells in the movement over the wound.

Chromatophores do not appear in the cutis until after two or three weeks, though regeneration begins here at the end of five days. In fact, the sub-epidermal part of the wound is filled with connective tissue before any chromatophores are to be seen in it.

It is therefore evident that the chromatophores of the regenerating epidermis cannot possibly come from the regenerating dermis. Dermal chromatophores are sometimes found at early periods, *i. e.,* after the fifth day, projecting slightly into the wound where they were probably carried passively by the advancing fibroblasts. They remain, however, near the margin of the wound.

Occasionally we found small cells bearing pigment granules in the fibrin or in the newly-formed connective tissue. They are leucocytes or young connective-tissue cells. The chromatophores of the dermis were not regularly arranged at the end of thirty-four days; they were missing at some places, and at other points they were situated deeper than is the case normally. They appear, occasionally, in increased numbers at the margin of the wound where they are sometimes surrounded by masses of small round cells.

In the experiments with wounds in tadpole skin, referred to previously in this paper, the regenerating tissue was taken at periods varying from a few hours to six days after the operation. The chromatophores of both the epidermis and the cutis showed characteristics like those that have just been described for the frog.

In the case of transplanted guinea-pig skin, pigment is produced by epidermal cells and is not directly the product of material carried to the

cell by the blood or lymph. An unpigmented cell is surrounded by the same nutrient as a pigment producing one; yet the latter only forms pigment. The pigment forming epithelium may be transplanted to a place where formerly unpigmented cells were situated, and it will continue to produce pigment, though the blood supply must remain the same. The production of pigment by these cells can in no way be compared to the formation of pigment in connective-tissue cells which are in contact with extravasated blood.

The evidence furnished by these studies against Ehrmann's hypothesis of the mesoblastic origin of epidermal chromatophores may be summarized as follows:

(1) We find no indications of a migration of pigmented or pigment-producing cells of any kind from the dermis into the epidermis.

(2) Chromatophores were observed to multiply by mitosis in the epidermis, and they are found regularly in regenerating epithelium long before any dermal tissue has regenerated in the space below.

SUMMARY.

1. Solutions of pilocarpine, atropine, and alcohol in which the animals lived constantly had little influence on regeneration.

2. The rapid movement of epithelium over the wound soon after cutting the skin is not due to cell proliferations. It is more probable that a tension, either previously existing or called into play by the wound, is the cause.

3. Cells divide both by mitosis and by amitosis in the regenerating epithelium. In regenerating frog epidermis mitoses are found in higher layers of cells than in guinea-pig skin.

4. Epithelial cells move in all directions into the sub-epithelial coagulum, and they may break through fibers of fibrin.

5. If the wound is large, the sub-epithelial clot may remain imperfectly organized, and some connective-tissue cells may degenerate. There is often very little regeneration of connective tissue below the wound as late as three weeks after the operation.

6. The chromatophores in the epidermis of frog skin behave in regeneration as ordinary epithelial cells and not as the chromatophores of the cutis. The former regenerate rapidly and the latter very slowly. During regeneration, epithelial chromatophores may be found in the coagulum underneath the epidermis.

7. There is no evidence of an ingrowth of chromatophores into the epidermis from the cutis, and the epithelium of the regenerating patch

of skin is fully pigmented before any pigment appears in the cutis below. The pigment of the epidermis is found in cells whose origin is strictly epidermal.

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THE CYTOPLASMIC AND NUCLEAR CHANGES IN THE STRIATED MUSCLE CELL OF NECTURUS.

BY

ALBERT C. EYCLESHYMER,

Professor of Anatomy, St. Louis University, St. Louis, Mo.

With 4 Plates.

The striated muscle cell, although the subject of a voluminous literature, still presents many problems worthy of investigation.

The unicellular character of the fibre has recently been questioned. The method of increase in the number of cells, the mode of growth of the individual cell, the origin of the sarcolemma are all problematic.

The cytoplasmic changes deserve renewed attention. The presence of fibrillæ in the living cell is denied by many. Those who regard them as veritable structures are not agreed as to their origin, their method of multiplication, or their extent in the cell. The character of the cytoreticulum, the arrangement of its meshes, and the relation of the fibrillæ to these meshes, should be further studied.

The nuclear changes especially merit extended investigation. The method of nuclear division is undecided; nuclear movements have been noted but not interpreted; nuclear structures, membrane, linin network, plasmosomes and karyosomes undergo striking changes in volume, position, and staining properties, but the significance of these changes is unknown.

The present study, while dealing to some extent with general problems in myogenesis, is devoted chiefly to the cytoplasmic and nuclear changes with the purpose of interpreting their relation during various phases of cytomorphosis.[1]

This work was begun in 1901 while the writer held an Austin Fellowship in the Harvard Medical School. To Professor Minot the writer is deeply indebted for guidance and encouragement. To Professor Barker,

[1] Minot, 01, 29, has used this word "to designate comprehensively all the structural alterations which cells, or successive generations of cells, may undergo from the earliest undifferentiated stage to their final destruction."

of the University of Chicago, he must also express his thanks for valuable suggestions and criticisms.

DETAILED DESCRIPTION OF SUCCESSIVE STAGES OF DEVELOPMENT.

The striated voluntary muscle cell of Necturus has been selected on account of the large size of its structural elements, and because a preliminary study of my own series of embryological stages, together with those in the Harvard Embryological Collection, assured me that a detailed study would yield new facts.

The material used was fixed in Flemming's stronger fluid, Zenker's fluid, corrosive-acetic acid, and picro-acetic acid. The nuclear stains employed were Delafield's hematoxylin, Heidenhain's iron-alum hematoxylin, alum cochineal and safranin. The cytoplasmic stains were eosine, orange G, and Lyons blue.

In comparing the various phases of cytomorphosis the cells have been studied in the same relative localities, as often as possible from the fifth or sixth post-aural segment. In comparing the nuclei the so-called resting stages have been selected. It should be stated here that the nuclei, up to the 26-mm. larva, divide by the typical indirect method. This statement must be qualified by the fact that thus far centrosomes have not been observed in striated voluntary muscle cells. As to the method of nuclear division in the later and adult fibres, little is known. Macallum, **87**, 462,[2] states that among the many hundreds of muscle nuclei which he examined, but a single case of division was observed and this one was found in heart muscle. I have likewise examined many nuclei with the hope of settling this point, but am as yet unable to say whether the division is direct or indirect.

When the myoblasts can first be distinguished by their cylindrical outlines, they are heavily laden with yolk granules (Fig. 1, *y*), which vary widely in size, but are quite uniformly distributed throughout the body of the myoblasts. At this time one often finds, in carefully teased material, cytoplasmic strands connecting the ends of myoblasts in adjoining myotomes (Plate I, Fig. 5, *c. s.*). These strands are numerous and indicate a widely extending syncytium.

Necturus 6-7 mm.—The anterior myotomes, which are now well defined, measure about 0.3 mm. in length. The axial portion or muscle

[2] The numbers indicate the year of publication and the page of the citation. No attempt has been made to give a complete bibliography, only those papers being listed which are not included in the extensive bibliography given by Heidenhain, 98.

plate is several layers of cells in thickness, and these cells or myoblasts extend from one end of the muscle-plate to the other, as can readily be demonstrated by teasing them apart. The peripheral portion or cutis-plate (Fig. 1, *c. p.*)[3] consists of a single layer of cells with their long axes radiating from the center of the myotome. The nuclei of these cells even at this early stage are distinctly different from those of the muscle-plate, not only in form and size, but also in staining capacity.

The yolk granules in the earlier stages were of uniform size and evenly distributed, indicating an even rate of absorption. At this time they are variable in size, the smaller being at the ends of the myoblast, where they grade off imperceptibly until they are no longer visible with the most efficient lenses.

This unequal rate of yolk absorption gives rise, as depicted in Figs. 1 and 2, to clearer zones at either end of the myoblast. A close study of these clearer ends, after osmic acid fixation, reveals the presence of a more or less distinct longitudinal fibrillation (Fig. 1). These cytoplasmic striations are more obvious on the notochordal side of the myoblast, and in many cases converge towards that side at the level of the inner margin of the clear zone. Whether these striæ are homogeneous or finely granular cannot be determined, since their structural analysis is beyond the definition of the best optical apparatus. In position and arrangement they correspond so closely to the fibrillæ, which are plainly defined in the stages immediately following, that one does not hesitate to assume that they are undifferentiated fibrillæ. Little is known of their origin. They may differentiate *in situ,* or they may represent lines of granules which, earlier scattered, have now become arranged in linear series. If the fibrillæ are first formed at the free ends of the myoblasts instead of along the side, as is usually held, the process of fibrillation would be quite in accord with the generally accepted theories of cellular differentiation (cf. E. B. Wilson, **96,** 40).

The nuclei of the myoblasts lie at different levels as shown in Fig. 1. In form they vary from the oval to the obtusely oval represented in Plate II, Fig. 14. Their average length as determined by many measurements is about 33 μ and their average width 10 μ. There are no indications of a paired arrangement; indeed they rarely lie opposite. Numerous mitotic figures are seen, but their spindles are always parallel with the long axis of the myoblast. It is thus obvious that neither in the posi-

[3] I have used the term cutis plate for convenience in designating the outer layer of the primitive myotome, but I do not wish to imply or express an opinion as to the fate of the cells of this layer.

tion of the nuclei nor in their plane of division does one find evidence of a longitudinal splitting of the myoblast.

The following description of the nuclear structures[4] is based upon the study of the nuclei both in teased preparations and in series of transverse sections.

The nuclear membrane (*n. m.*) is here and there obscured by a layer of karyosomes (Plate II, Figs. 21, 22, 25, 26), but is in general well defined. In places the cytoplasmic reticulum appears as threads terminating in the membrane.

The linin network (*l*) is of fairly uniform character in all the preparations. The large open spaces seen in some of the sections (Figs. 18, 21, etc.) are artifacts due to imperfect cutting. The threads of this delicate network pass into the nuclear membrane, but I have been unable to find any indications of a continuity between them and the cytoplasmic reticulum; the latter is not only poorly defined, but also less deeply stained by nuclear dyes, leading one to regard the linin network and cytoplasmic reticulum as fundamentally different.

Three or four plasmosomes (*pl.*) are usually present in each nucleus. They are rarely found at the periphery, but otherwise show no constancy in position; they are usually spherical, fairly uniform in size, surrounded by a sheath of deeply staining granules, but themselves possess only slight affinity for nuclear stains.

The karyosomes (*ky.*) shown in Plate II are numerous and variously distributed in the different nuclei. In many there is a peripheral zone which is comparatively free from these structures. In some there is no particular arrangement with reference either to periphery or axis. They are usually irregular in form and size, with numerous processes which extend along the linin threads and form anastomoses with adjoining karyosomes.

Necturus 7-8 mm.—As development proceeds, the clear zones at either end of the myoblast become wider, owing to the continued absorption of the yolk granules, and one is thereby enabled to make out more definitely the relation of the striated or fibrillated tract to the other portions of the cell. The fibrillæ have extended farther along the notochordal side of the cell and appear as represented in Fig. 4, Plate I, being grouped in brush-like form with the point of convergence (*a*) at the level of the inner margin of the clear zone (*c. z.*). I have repeatedly looked for centrosomes at the points of convergence, but without success. The

[4] In describing the changes in nuclear structures I have adopted the terminology used by E. B. Wilson, 96.

fibrillæ do not as yet show any indications of transverse bands or markings.

Necturus 8-9 mm.—This stage, which is well illustrated by Fig. 8, shows an extension of the fibrillated tract. The apparent apex of the cone or brush is not far removed from the place at which it was located in the preceding stage. The converging fibrillæ, however, no longer terminate here, but continue in a closely aggregated bundle or tract throughout the length of the myoblast, and again spread out into a similar brush or cone at the opposite end. To make the description more complete, it should be remarked that at each end of the myoblast a conical group of fibrillæ is formed and these extend from either end towards the middle until they meet and form a continuous tract. Where a considerable number of the fibrillæ are close together, as represented in Fig. 8, the transverse markings are now plainly seen, but as they spread out in small groups or singly, these markings are more obscure and are indicated only in the moniliform outline of the fibril.

This peculiar arrangement of the fibrillæ, in the myoblasts of Necturus, is similar to that described and figured by Heidenhain in the fibrillation of the ciliated cells in the hepatic ducts of Helix and in the intestinal epithelium of the frog, where the fibrillæ take on a conical arrangement with their apices on one side of the cell. An almost identical arrangement of the fibrillæ has been found by Godlewski, **02**, in the myoblasts of the rabbit, in which form he states that the fibrillæ of one myoblast extend over into the myoblast of an adjoining myotome. This relation I can positively say does not exist in Necturus.

Necturus 9-10 mm.—The myotomes in the post-aural region have now reached a length of about 0.4 mm. and present the general appearance shown in Plate I, Fig. 2. The clear zones at either end of the myoblast are wider; the fibrillated tracts are considerably increased in diameter, and the notochordal sides of the myoblasts are better defined. In the sections it is not difficult to trace the outlines of the myoblasts from one end of the myotome to the other and to see that now, as in the earlier stages, the ends of the myoblasts in adjoining myotomes are frequently connected by cytoplasmic strands.

Transverse sections of the myotome at this time show a well-defined cell membrane which in teased preparations is usually ruptured. Since there are as yet no mesenchymal cells, either between the ends of the myotomes or among the myoblasts, this membrane must represent whatever sarcolemma the myoblast now possesses.

Necturus 12 mm.—Previous to this time there is no indication of the

formation of the septa and endomysium, but the beginnings are now indicated by the migration of a large number of mesenchymal cells into the spaces between the ends of the adjoining myotomes, as shown in Fig. 3 (*mes.*). These cells emanate from two sources; the greater number are derived from the peripheral mesenchyme; in addition to these a considerable number come from the axial mesenchyme, as described by Maurer, **92**, in Siredon. These cells not only give rise to the septa, but also wander in among the myoblasts and eventually invest each myoblast with a more or less complete sheath of connective tissue which forms a part of the sarcolemma or becomes intimately associated with it.

Many karyokinetic figures are now observed in and among the muscle fibres; these are in part those of the mesenchymal cells, and may have their spindles in almost any plane. It is therefore necessary to use considerable care in the interpretation of the various figures lest they be confused with the nuclei of the myoblasts, in which the planes of division are always parallel with the long axis of the cell.

Through the absorption of the yolk, the character of the cyto-reticulum is now clearly revealed, so that its relation to the fibrillæ is more readily determined. Various methods have been employed to bring this reticulum into prominence, especially Kolossow's osmic acid method. The meshes of the reticulum, as shown in Fig. 9, are exceedingly variable, and bear no fixed relation either in form or size to the fibrillæ.

The structure of the nuclei at this time is not widely different from that described and figured in the 6-7 mm. embryo. Since the changes taking place are more pronounced in the 15-17 mm. larvæ, their further description is here unnecessary.

Necturus 15-17 mm.—The myotome, which has now (Fig. 6) attained a length of about 0.5 mm., appears quite unlike that of the earlier stages. This change has been brought about by the continued invasion of mesenchymal cells. These cells have sent out long cytoplasmic processes which have so intertwined that they form a distinct connective-tissue septum (*s*). In the earlier stages these mesenchymal cells contained large nuclei with a small quantity of cytoplasm and were actively migratory; they now contain small nuclei with greatly elongated cytoplasmic processes and are no longer migratory. With little difficulty one can follow all the intermediate stages and see that the connective-tissue fibrils are the elongated cytoplasmic processes of the previously wandering cells. The yolk granules have been rapidly absorbed, until but few are present in the cytoplasm. *Pari passu* with the absorption of the yolk the fibrillæ have greatly increased in number, as shown in Fig. 11, so that one-half or more of the cell is fibrillated.

Transverse sections show that the bases of the so-called brushes or cones may take on different forms, as represented in Fig. 11. In this figure the two myoblasts on the right exhibit the patterns often found, while the three on the left represent forms less frequently seen. The condition pictured in the lowest myoblast might lead to the supposition that there is a peripheral band of fibrillæ, but in all cases of this kind the study of serial sections shows that the fibrillæ converge on the notochordal side of the myoblast.

If a transverse section through the end of a given myoblast be compared with a like section through its middle, it will be found that the number of fibrillæ in the former is greatly in excess of those in the latter. This fact is in harmony with the assumption that the fibrillæ increase in number through longitudinal division.

The nuclei have increased in number until four and often five are found in each myoblast. Transverse sections of this stage show that they no longer occupy an axial position, but are eccentrically placed, often lying close against the outer side of the myoblast, in a position intermediate between that shown in Figs. 11 and 12. The nuclei have a somewhat different outline from those of the early stages, in that they are longer and more pointed, as shown in Plate III, Fig. 27. The nuclear membrane in many nuclei is lined by a layer of karyosomes, which is so closely applied to the membrane as to appear inseparable from it. The meshes of the linin network are larger, with a noticeable increase in the diameter of the threads. The plasmomeres have decreased in number, and in many nuclei have disappeared entirely.

The karyosomes are in general more numerous and larger than in any of the earlier stages. They are irregularly scattered throughout the nucleus, and vary in form from round to elongated masses with numerous processes extending along the linin threads. In some cases they are arranged in two or three irregular rows which extend, in a general way, parallel with the long axis of the nucleus. Frequently there is found a nucleus with a marked axial aggregation of karyosomes, but in the greater number there is a tendency towards a peripheral condensation.

Necturus 21-26 mm.—In the larvæ of these lengths the myotomes measure about 0.6 mm. and show a corresponding increase in diameter. The myoblasts are not notably different in general character from those of the preceding stages.

The peculiar arrangement of the yolk granules observed at this time (Fig. 7) leads me to remark that in the early stages the myoblasts possess an enormous number of large yolk spheres which often equal the nucleus in size. As development proceeds these spheres become broken

up into smaller spheres, which are gradually absorbed. This process continues, until in the 21 mm. stage, the spheres or granules that remain are very minute and often arranged in rows. If the 26 mm. stage be examined after osmic acid fixation these blackened granules stand out with great clearness. They are now disposed in regular linear series and in correspondence with the transverse markings. These granules frequently lie at the surface of the myoblast and again within; often they terminate at the ends of a nucleus, appearing as if in continuity with the nuclear substance.

Similar structures have been repeatedly described in adult muscle cells of various amphibia. By Kölliker, **57**, they were considered as a third normal element of the muscle cell. Max Schulze, **61**, regarded them as an undifferentiated portion of the primitive protoplasm; while Weber, **74**, and van Gehuchten, **89**, concluded that they were pathological structures. I have not found these structures in the adult fibre of Necturus. Their structure and arrangement in the late larval stages have suggested that possibly there is a close relation between them and the problematic structures described by others in the adult fibres.

The nuclei lie at the periphery of the myoblast, and for the most part on the cutis side (Plate I, Fig. 12). In general form they are somewhat longer than in the earlier stages, attaining an average length of about 47 μ and a width of 10 μ; they are also more or less flattened, as shown in Plate III, Figs. 28-39, thereby bringing a much greater extent of nuclear surface in contact with the cytoplasm.

The profile view and transverse sections represented in Plate III show that the nuclear membrane (*n. m.*) is more completely obscured by the peripheral layer of karyosomes than in the earlier stages.

The linin network (*l*) has undergone striking changes in character. It is greatly decreased in quantity; its threads are coarser, more regular, and in general radially disposed.

One of the most striking changes is the entire disappearance of plasmosomes which are present in both the earlier and later stages.

The karyosomes, as shown in Plate III, Figs. 27-39, are arranged in a more definite manner than in the preceding stage, being usually so grouped that they form an irregular axial mass and a wide peripheral band. Those in the axial mass are larger than those at the periphery and are most frequently so disposed that their long axes are parallel with the long axis of the nucleus. Those at the periphery are so closely apposed that they give rise to an apparently continuous band, but this peripheral band, instead of being of uniform width, is much thicker on the side next the fibrillæ.

It is important to note that the type of nucleus above described is most frequently found, in the 26 mm. stage, at the upper and lower margins of the myotome. The myoblasts in these localities are less densely fibrillated than elsewhere and are to be considered, as many writers have maintained, the more recently formed myoblasts.

Necturus, 23 cm. (adult).—In the adult of this length the cephalic muscle segments, and consequently muscle cells, measure 4.0 cm. The area of their transverse section is greatly increased as a comparison of Figs. 12 and 13 will clearly show. The cells are separated by a relatively large quantity of connective tissue or endomysium. The muscle cells in transverse sections present somewhat different appearances; in those more frequently observed (Plate I, Fig. 13), the fibrillæ appear evenly distributed and have a considerable sarcoplasm among them; the less common appearance is that seen in the contracted muscle cells, in which the fibrillæ are more closely apposed, giving to the cell a denser appearance.

The nuclei have again shifted their position. They are no longer situated at the periphery of the cell, as in the 26 mm. stage, but are, for the most part, evenly scattered throughout the sarcoplasm, as shown in Fig. 13. They have also changed somewhat in form, being longer, wider, and flatter. The most striking difference seen when the nuclei of the early and adult stages are compared is the faint staining of the latter.

The description of the nuclear structures, as shown in Plate IV, is based upon the study of both isolated nuclei and series of transverse sections. A series of transverse sections of an adult nucleus is represented in Figs. 42-61. In structure this nucleus is typical, but in form it is less flattened than usual. The nuclear membrane is visible throughout the greater portion of its extent with small karyosomes occasionally in contact with it. In some sections, such as shown in Figs. 50, 51, there appears to be a wide layer of karyosomes lying against the membrane. But it will be shown later that this apparent band is in reality due to an infolding of the membrane. I was at first unable to account for these peculiar appearances, which were never seen in profile views (Fig. 41); however, it was later observed that these nuclei are always found in fibres which appear much denser in internal structure. This appearance is due to the contraction of the fibrillæ, which increase in diameter and become closely apposed. Through the pressure thus brought about the nuclei become deeply serrated, and when viewed from the end or in transverse section this infolding of the membrane produces the effect

shown in transverse section. These surface serrations were first observed by Weber, **74**, 489, in the nuclei of the muscle fibres of the frog and correctly interpreted as the result of pressure from the muscle fibrillæ.

I was later gratified to find that Macallum, **87**, had previously studied the nuclear membrane in these particular nuclei. The nuclei were isolated from the muscle fibre of the adult Necturus by macerating in formic acid and then stained in gold chloride. By this method Macallum was able to demonstrate both a longitudinal and a transverse striation of the nuclear membrane, which he attributed to the pressure of the fibrillæ.

The linin network is seen more distinctly in transverse sections (Figs. 42-60) than in the profile. Its meshes are smaller than in the 17-26 mm. stages; in some sections large, irregular meshes are seen (Figs. 48, 55, 58), which are due to the rupture of a number of threads during preparation. The threads are finer than in the 17-26 mm. stages and show decidedly less affinity for chromatic stains. Macallum says that " there is in some nuclei a reticulum like in every respect to that found in muscle substance." My studies, however, lead to the conclusion that the network in the nucleus is entirely independent of that in the cytoplasm.

Plasmosomes are always present, but in general are fewer than in the 6-15 mm. stages; most frequently they are distributed somewhat as in Fig. 41. There is no marked sheath of chromatic granules around them and their staining capacity is notably less than in the earlier stages.

The karyosomes, as shown in the various figures in Plate IV, are remarkably few in number when compared with any of the preceding stages; they are smaller, usually lie within the membrane, rarely if ever uniting in a peripheral band. The most striking difference is their slight affinity for nuclear stains. Macallum found many nuclei in which there was no chromatin. Although I have made a careful examination of a very large number of nuclei, I have never found one in which there was no chromatin; it seems quite probable, however, that such may occur. At any rate the important fact should be emphasized that one of the most striking characters of the old nuclei is a great reduction in the amount of chromatin.

GENERAL DISCUSSION OF VARIOUS PROBLEMS IN MYOGENESIS.

A. Concerning the Myoblasts as a Whole.

ITS UNICELLULAR CHARACTER. METHOD OF INCREASE IN NUMBER AND SIZE OF THE FIBRES. THE SARCOLEMMA.

The revival by Godlewski of the older theory that the muscle fibre is a multicellular structure, as first formulated by Valentine and Schwann,

is based first upon the fact that in the rabbit embryo there are cytoplasmic bridges between the ends of the myoblasts of the adjoining myomeres, and secondly upon the observation on the continuity of the fibrillæ through these bridges.

In the stages of Necturus immediately preceding the differentiation of myoblasts there are numerous anastomosing cytoplasmic processes among the mesothelial cells, indicative of a widely extending syncytium. When the myoblasts can be distinguished by their cylindrical outlines, they are heavily laden with yolk granules, yet it is not difficult to find, in carefully teased preparations, cytoplasmic strands connecting the ends of these myoblasts. As soon as the yolk is absorbed in the ends of these myoblasts (6-8 mm. embryo) the connecting strands are more clearly defined in both teased preparations and longitudinal sections. From this stage up to the 10 mm. embryo, when the mesenchyme has grown in to form the septa, cytoplasmic continuity between the ends of the myoblasts is frequently observed. After the septa are formed and the myoblasts fibrillated (17-26 mm.), I have been unable to trace their continuity.

While there is a more or less complete syncytium of the myoblasts in the early stages, there is no evidence whatever in Necturus to support Godlewski's view that the muscle fibre is formed through an end-to-end union of the myoblasts in adjoining myotomes. Indeed, strong evidence against this view is found in the fact that in each of the closely connected stages, from the formation of the myoblasts up to and including the 26 mm. larva, the myoblasts may be easily isolated. Exact measurements show that the length of each corresponds precisely to the length of the myotome from which it was taken. There is not the slightest evidence that the fibrillæ of one myoblast are continued into another myoblast.

As to the mode of increase in the number of fibres, I think it can safely be asserted that the majority of investigators believe that it is by the differentiation of new myoblasts around the margin of the myotome. It was claimed by Kühne, **71**, Goette, **75**, Bremer, **83**, Kölliker, **88**, and others, that the increase takes place not only in this way, but also by the post-embryonic formation of inter-muscular spindles. The later investigations, however, indicate that these are the endings of sensory nerves. The sarcoplasts, which were considered by Margo, **59**, and Paneth, **85**, as embryonic muscle cells, have later been interpreted by Felix, **88**, S. Meyer, **86**, Bardeen, **00**, Godlewski, **02**, and others, as degeneration products. Others have held that the fibres increase in number through

longitudinal splitting; this was advocated first by Remak, **43**, and more recently by Felix, **88**, and Godlewski, **02**.

In Necturus the increase in the number of muscle cells in the postaural myotomes proceeds at about the following rate: In the embryo of 10 mm. there are about 50 muscle cells; in the 15 mm., 150; in the 21 mm., 500; in the 26 mm., 1500. During these phases of rapid increase the cells have been carefully and repeatedly examined with a view of finding out whether or not a longitudinal splitting occurs. In no instance have I found a myoblast undergoing division, nor have I found, either in the position of the nuclei or in their direction of division, any evidence that they participate in the division of the myoblasts.

The increase in number is most pronounced at the dorsal and ventral margins of the myotomes, yet new myoblasts are being formed on both the lateral surfaces. The addition of new myoblasts is continued long after the earlier formed muscle cells are fibrillated; in this respect the condition in Necturus differs somewhat from that described by MacCallum in the pig and man, in which the increase in the number of fibres ceases at the time the first-formed myoblasts are fibrillated. The sole method of increase between the 10 mm. and 26 mm. larva, so far as I have been able to observe, is by the differentiation of new myoblasts around the periphery of the myotome.

Regarding the increase in size of the muscle cell, want of material precludes more than a preliminary statement. Up to and including the 26 mm. larva, there are no indications that an increase in size is brought about through the lateral fusion of the myoblasts which, according to Godlewski, does occur in the rabbit. The increase in size appears to be due to the continued formation of the fibrillæ. As pointed out in a preceding page, careful counts show that the number of fibrillæ in the ends of the myoblasts is greatly in excess of the number at the middle of the myoblasts. That this is due to a longitudinal splitting rather than new formation is supported by the fact that teased preparations show many fibrillæ divided along a portion of their extent. Again, if new formation plays any considerable part there would be found short fibrillæ without transverse markings, but such are not found. From these observations, I am led to believe with Apáthy, **92**, Maurer, **94**, and Heidenhain, **99**, that the increase in number of fibrillæ is due to growth and longitudinal division.

Since its discovery by Valentine the sarcolemma has been the subject of repeated study. While much has been written there is as yet the widest divergence of opinion. Schneider, **87**, says it is an artifact. Wage-

ner, 69, considered it a sheath of connective tissue. Deiters, 61, Bremer, 83, and others, have regarded it as a cuticularized portion of the cell. F. E. Schulze, 62, Kölliker, 88, and others, maintain that it is the cell-membrane.

The cell-membrane is easily distinguished up to the time the mesenchyme grows in and becomes closely applied to it. Either the cell-membrane is the sarcolemma or the cell possesses no sarcolemma in its earlier stages.

In the later stages one frequently finds the myoblasts so contracted that their ends have drawn away from one or both septa. In such cases the endomysium and sarcolemma remain attached to the septa, and it is not difficult to discern two entirely different structures; the outer, a fibrous sheath made up of several layers; the inner, a delicate membranous sheath which, I believe should be considered as the sarcolemma.

B. Changes in the Cytoplasm of the Myoblast.

Character of fibrillæ. Method of fibrillation. Character of cyto-reticulum. Relation of fibrillæ to cyto-reticulum.

Many and varied have been the hypotheses offered to explain the formation and structure of the fibrillæ. By some of the earlier writers (Deiters, Rouget) they were regarded as extra-cellular products. So far as I am aware no one would now question the generally accepted opinion that they are intra-cellular and are formed in the cytoplasm. It should not be forgotten, however, that Robin held that they arise from the free ends of the nuclei by a process of gemmation, a view which was later supported by both Retzius, 81, and Bremer, 83.

As to the nature of the fibrillæ, there are at least two current views. The first is that in the normal fibres there is neither cyto-reticulum nor fibrillæ, these apparent structures found in the fixed tissue being coagulation products. This view was ably advocated by Englemann, 70 to 80, and has since been supported by many physiologists. The second is that there are differentiated structures, network or fibrillæ, or both, in the living myoblasts. The latter view is that accepted by the greater number of histologists.

The so-called "network theory" took its remote origin from the discovery by Bowman, 41, that the muscle fibre could be cleft both longitudinally and transversely, giving rise to the sarcous elements. Jones, 44, and Dobie, 48, held that these elements were united end to end by a cementing substance, while others claimed the existence of a like substance between the sides of the sarcous elements. Thus modified the theory

was accepted by a great number of workers, among whom were: Remak, 43, Harting, 54, Haeckel, 57, Munk, 59, Margo, 59, and Krause, 68. A decided advance was made when Thin, 76, found that after gold chloride staining the cementing substance of the earlier writers was revealed as a network which Thin considered the contractile part of the muscle-cell. This theory was elaborated by Retzius, 81, Bremer, 83, Carnoy, 84, Melland, 85, Marshall, 87 and 90, van Gehuchten, 88, and Ramon y Cajal, 88, all of whom maintained that the muscle cell contains a contractile reticulum, the meshes of which were filled with a more fluid substance. By some the longitudinal threads of this network were interpreted as fibrillæ. Others held that the fluid substance in the meshes of the reticulum coagulated through the action of various agents and thus formed the fibrillæ.

The fibrillar theory was established through the early investigations of Prevost and Dumas, Treviranus and Berres, who regarded the fibrillæ as homogeneous structures. The theory was supported by Schwann, 39, Henle, 41, Gerlach, 48, Kölliker, 50 to 00, and others. During recent years it has received further support through the investigations of Rollet, 85 to 91, Eimer, 92, Schäfer, 91, Rutherford, 97, McDougall, 97, Heidenhain, 99, and Godlewski, 02, all of whom regard the fibrillæ as the contractile elements and as arising independently of the cyto-reticulum. As to the exact nature of the fibril, however, there are different opinions. Rollet, Heidenhain and Godlewski consider the fibril as a semifluid homogeneous structure, while Schäfer, McDougall and others regard it as regularly segmented and hollow in certain portions.

A potent argument against the coagulation hypothesis is the fact that the fibrillæ have been repeatedly observed in the living fibre. Sachs, 72, and Wagener, 73, observed them in the wing-muscles of insects; Kiefer-stein, 59, and Kölliker, 66, in petromyzon; Hensen, 68, and many others in the frog; Frederique, 75, in mammals.

I have repeatedly observed the fibrillæ in the living muscle cells of the larval Necturus. A study of the fresh material in normal solutions shows that the fibrillated portion of the cell is of the same extent as in the fixed and stained material. Not only is this true of the different stages of growth, but furthermore, in the same embryo, one can readily follow the decreasing diameters of the fibrillated tracts from the mid-dorsal to the caudal myotomes. A point of capital importance is found in the fact that in Necturus, Amia, Lepidosteus, as my own observations show, and in other forms, as Kaestner, 92, has found, the beginning of fibrillation is coincident with the first contractions. The movements of

the embryo first begin in the anterior of the mid-dorsal myotomes and in these the myoblasts are first fibrillated. The above considerations lead the writer to support the theory that the fibrillæ are pre-existent structures and represent the principal contractile elements.

MacCallum, 98, has found that in the myoblasts of pig and of man there is a primitive cytoplasmic reticulum, the meshes of which later assume a regular form; the transverse membranes of this meshwork eventually form the so-called Krause's membranes, while the longitudinal give rise to the fibrillæ. I quote the author's words (p. 211): "It simplifies the conception of the structure of striated muscle fibre greatly, to consider the fibril bundles and the membranes bounding the compartments in the sarcoplasm as derived from the primitive network found in the muscle cells of very young embryos" (p. 209). "This network tends to become more and more regular until the meshes are of the form of large discs. Some of these break up into smaller ones, and in the nodal points of the network there is an accumulation or differentiation of its substance, giving rise to longitudinally disposed masses. These become what in the adult are known as fibril bundles and the discs are the sarcoplasmic discs."

While MacCallum's theory is exceedingly ingenious and strongly appeals to those who would reconcile the network and fibrillar theories, I cannot see, at present, how it will explain the facts observed in the fibrillation of the muscle cell of Necturus.

In the study of the muscle cell of Necturus I have been unable to find any evidence of a definite or fixed relation between the cytoplasmic network and the fibrillæ. It seems highly improbable that the longitudinal threads or membranes of such a meshwork should converge at the notochordal side of the myoblasts, which would be necessary if the fibrillæ differentiated in its meshes. Further, in conformity with the subdivision of the muscle columns, the meshes must become progressively smaller towards the end of the myoblasts, as a result of their repeated subdivision. Even were this true, a further difficulty is encountered in the fact that the cytoplasmic reticulum varies widely in the different myoblasts, and in different portions of the same myoblast. Another serious objection is the fact that the fibrillæ are unstriated for some time after their first appearance. My observations agree with the views already expressed by Wagner, 69, Rabl, 97, and Bardeen, 00, all of whom maintain that the fibrillæ are at first without transverse markings. Further, it should be borne in mind that some of the most recent investigators (Godlewski, 02) have been unable to find any evidence of such a network in the mam-

malian embryo. In view of the above facts I am led to the conclusion that the fibrillæ in the myoblasts of Necturus bear no fixed or definite relation to the cytoreticulum.

C. Changes in Nuclei of Muscle Cell with Reference to Changes in Cytoplasm.

Relative volumes of nuclear and cytoplasmic material. Movements of nuclei with reference to areas of cytoplasmic activity. Changes in structure of nuclei.

It is necessary to know the average sizes of the nuclei in the successive stages of development before a comparison of the volumetric relations of nuclear and cytoplasmic material can be made. The measurements given in the following table were made from nuclei in teased fibres. Although but ten measurements are recorded, these are typical of a much more extended series.

	7 mm.		10 mm.		17 mm.		26 mm.		23 cm.	
1.	30	12	35	10	43	10	56	10	53	7
2.	30	10	35	11	45	8	36	15	65	9
3.	28	10	32	13	49	10	45	12	70	9
4.	35	10	28	9	42	9	42	11	49	10
5.	30	13	37	12	48	8	46	8	62	11
6.	25	9	31	11	46	7	40	10	56	10
7.	33	8	36	12	42	8	57	11	60	5
8.	33	9	28	13	44	7	54	13	52	7
9.	37	11	37	13	48	10	37	9	40	6
10.	30	12	30	14	44	9	58	9	56	8
	—	—	—	—	—	—	—	—	—	—
	33.1	10.4	32.9	11.8	45.1	8.6	47.3	10.8	56.3	8.2

The numerals at the left represent the serial number of the nucleus measured. The numbers in millimeters above the columns give the lengths of the embryos compared. The first column shows the length of the nucleus in microns; the second the width. The numerals below these columns express the average length and width of the nuclei in the respective embryos.

Since the nuclei are irregular in outline any estimate of their volumes must be subject to considerable error. It will be seen by glancing at the above table that the nuclei in the successive stages increase in length, but that this is counterbalanced to a certain extent by a decrease in diameter. It has therefore been thought best to consider the nuclei as uniform in size and as representing a unit of volume. In computing the volume of the cytoplasm the cells have been isolated and their lengths and diameters measured. Although they are not of uniform diameter

throughout, nor always cylindrical, it has been assumed that they represent perfect cylinders. The results obtained will be far from exact, yet they must represent approximately the relative conditions. Moreover, it may be said that no other cells in the body are more regular or admit of more precise measurement. In determining the relative volume of cytoplasmic to nuclear material I have used the following formula:

$$\frac{\text{Radius}^2 \times 3.1416 \times \text{Length of muscle cell}}{\text{Number of nuclei}} = \text{Ratio of cytoplasmic volume to nuclear volume.}$$

Length of muscle cell.	Diam. of muscle cell.	No. of nuclei in muscle cell.	Amount of cytoplasm (in cu. mm.) per nucleus.
	8 mm. Embryo.		
0.4 mm.	.01 mm.	1.	.00003141
0.4 mm.	.01 mm.	2.	.00001570
0.35 mm.	.01 mm.	1.	.00002748
0.38 mm.	.01 mm.	1.	.00002985
0.4 mm.	.01 mm.	1.	.00003141
	17 mm. Embryo.		
0.5 mm.	.02 mm.	3.	.00005236
0.5 mm.	.02 mm.	4.	.00003927
0.5 mm.	.01 mm.	2.	.00001964
0.4 mm.	.02 mm.	3.	.00004180
0.4 mm.	.02 mm.	2.	.00006283
	26 mm. Embryo.		
0.6 mm.	.04 mm.	8.	.00009424
0.6 mm.	.02 mm.	3.	.00006283
0.6 mm.	.04 mm.	9.	.00008377
0.5 mm.	.04 mm.	8.	.00007854
0.5 mm.	.06 mm.	15.	.00009424
	23 cm. Adult.		
3.6 mm.	.12 mm.	170.	.00023925
4.0 mm.	.15 mm.	200.	.00035343
4.2 mm.	.10 mm.	160.	.00020616
2.5 mm.	.08 mm.	100.	.00012566
4.0 mm.	.10 mm.	150.	.00020944

The above computations show interesting changes in the relative volumes of cytoplasmic and nuclear material during growth. In the 8 mm. embryo a unit of nuclear material is correlated with two to three units of cytoplasm; in the 17-26 mm. embryo with five to seven units of cytoplasm; in the adult with twenty to thirty units of cytoplasm. In other words, as the embryo approaches the adult condition there is a progressive increase in the amount of cytoplasmic material with the end result that there is twenty to thirty times as much cytoplasm in physiological equilibrium with a given quantity of nuclear material as in the earlier

stages. Minot, in 1890, called attention to this feature of cell life in the following words: "In all the principal tissues of the body we meet everywhere the same phenomenon of growth, namely, that with the increasing development of the organism and its advance in age we find an increase in the amount of protoplasm. We see that there is a certain antithesis, we might almost say a struggle for supremacy, between the nucleus and protoplasm.

"We have then to state, as the general result of the studies which we have just made, that the most characteristic peculiarity of advancing age of increasing development, is the growth of protoplasm; the possession of a large relative quantity of protoplasm is a sign of age."

Whether this disparity in the muscle cell is due entirely to an increase in the amount of cytoplasm, or whether there is in the later stages a reduction in the amount of nuclear material, is uncertain. Bowman, as early as 1844, said: "It is doubtful whether the identical corpuscles (nuclei) originally present remain through life, or whether successive crops advance and decay during the progress of growth and nutrition." Kaestner, 90, 6, found that the nuclei in the muscle cells of the duck elongate, become smaller and smaller until finally, in a very late stage, they disappear. Maurer, 94, 580, states that the muscle nuclei in Siredon occupy at first a central position, later a part of them migrate to the periphery, but those remaining undergo degeneration and disappear.

While I have found no evidence of complete disintegration of the nuclei, I am unable to say that it does not occur. The fact that in the older stages the nuclei possess but little chromatin suggests that complete chromatolysis may occur.

It has long been known that the nuclei of the muscle cell undergo striking changes in position during growth, but the meaning of these movements has been scarcely considered. The only suggestion which merits quoting is that by MacCallum, 98, 211, who finds that in the human embryo of 130-170 mm. the muscle fibres stop increasing in number and that at this time the nuclei change in character from the vesicular, centrally disposed, to the solid, peripherally placed, nuclei. MacCallum suggests that there is possibly a relation between the position of the nuclei and the power of the cells to produce new fibres.

As pointed out in the descriptive portion of this paper, the nuclei occupy an axial position at the time the first fibrillæ are formed; as the differentiation of fibrillæ continues, from the inner toward the outer side of the cell, there is a corresponding movement of the nuclei toward the outer side. When the cell is completely filled with fibrillæ the nuclei

are found, almost without exception, on the outer side of the cell. In the adult fibres the nuclei are no longer found exclusively at the periphery, but are scattered throughout the sarcoplasm and show no definite arrangement either with reference to the planes of the animal or to the axis or periphery of the cell itself. Why these nuclei come to lie within the muscle cell is unknown. It is possible that with the continued growth of the fibre their sphere of activity becomes too far removed from that of cytoplasmic activity, necessitating a redistribution of nuclear material.

The nucleus thus undergoes a series of striking changes in position which correspond to, if they are not correlated with, the shifting areas of cytoplasmic activity. It is possible that the movement of the nucleus from the axis of the cell to the periphery is the result of mechanical factors, in that the continued formation of fibrillæ from the inner toward the outer side of the cell would cause a corresponding displacement of the nucleus. To account for their later position among the fibrillæ through the influence of mechanical factors is exceedingly difficult, but becomes intelligible if we regard the movements as of physiological significance.

There are many observations which seem to show conclusively a physiological correlation between nuclear movements and cytoplasmic activity. The most familiar instances are found in the various gland cells to which nearly all histologists have called attention. In other animal cells the same phenomena have been observed. I need but cite here the work of Korschelt, **89,** who found that in the ovarian eggs of a large number of insects the nucleus moves toward the locality at which the egg receives its nutriment and which must be interpreted as the area in which the cytoplasmic activity is greatest. Some striking instances of like movements are to be found among plants. Haberlandt, **87,** from an extended study of nuclear movements, concludes that the nucleus moves to the area of greatest cytoplasmic activity, where it remains until the period of local activity ceases, when it returns to its original position. A beautiful illustration is found in the growth of epidermal hairs. In the root hairs, where the growth is terminal, the nuclei are found at the ends of the cells, but in the aërial hairs, where the growth takes place at the base, the nuclei are found at the base of the cells. Tangl, **84,** observed that in the scales of Allium sepa the nuclei always gather at the points where the cells have been injured. The same movements were observed in Vaucheria by Haberlandt, showing that in regeneration the nuclei likewise migrate to the areas of accelerated cytoplasmic activity.

These and many other observations indicate that the position of the nucleus in the muscle cell, as in other cells, is not one of an accidental character, but one brought about through a precise physiological correlation of cytoplasmic and nuclear activities.

The changes in the structure of the nuclei may be summarized best by considering separately the various nuclear elements.

The nuclear membrane shows some interesting changes during cytomorphosis, the most notable being the formation of grooves or corrugations in the adult stages which serve to increase considerably its contact surface. Chemical changes are also indicated by its decreased staining properties.

The linin network in the earlier stages (6-15 mm.) is made up of fine meshes, which are more or less obscured by the numerous and widely scattered karyosomes. As the muscle cell approaches the period in which its cytoplasmic activity is most marked the meshes of the nucleus become larger and the threads straighter and coarser, with increased staining capacity. As it passes over into the adult condition the fine meshwork reappears but the affinity for chromatic stains has been lost.

The plasmosomes show interesting changes during the growth of the myoblast. In the earliest stages (6-7 mm.) but two or three are found, but as the differentiation of the cytoplasm proceeds (9-10 mm.) they become more numerous, four or five being usually present. With the increased cytoplasmic activity of the 17-26 mm. stages these structures entirely disappear, but later reappear in the old nucleus. In the early stages they readily stain with any of the ordinary basic stains, but in the old fibre this capacity is greatly lessened if not lost. From my observations I am unable to offer evidence either for or against the supposition that these structures are to be regarded as by-products rather than active nuclear elements.

Concerning the arrangement of karyosomes or chromatin in the nuclei of the muscle cells of Necturus there is a single observation to be cited. Macallum, **87**, 462, says: "In what might be considered as young nuclei the chromatin is usually quite distinct, arranged in short, variously looped pieces along the long axis of the nucleus, or in the form of minute nodules (nucleoli) in different positions in the nuclear cavity."

Rabl, **89**, 242, describes the muscle fibres in the embryo of Pristiurus at the time the first fibrillæ are formed, and in speaking of their nuclei says that in a very early stage they show peculiar characters; they stain less intensely than the nuclei of the surrounding mesenchymal cells and possess an axial rod of chromatin.

Minot, 92, 474, finds the "same peculiarity in the chick, but later the nuclei lose this main granule and have instead a number of smaller ones."

The changes in the quantity and quality of the chromatin during the growth of the muscle cell is more striking than the changes observed in the other nuclear structures. In the early stages (6-7 mm.) the karyosomes are comparatively small, and quite evenly scattered through the nucleus; soon, however (10 mm.), the chromatin shows a tendency to aggregate in large karyosomes, which are irregularly disposed. These large masses of chromatin then (17-26 mm.) become grouped in the axial and peripheral portions of the nucleus, the peripheral layer being much thicker on the side of the nucleus, which is applied to the fibrillated surface of the myoblast.

This series of changes resulting in the greatly increased quantity of chromatin goes on hand in hand with the increased cytoplasmic activity manifested by fibrillation. Another striking change in the nucleus during the phases of greatest cytoplasmic activity is the entire absence of plasmosomes. We find in these changes a most remarkable and perfect correspondence to the changes known to occur in the nuclei of gland cells during phases of activity. I quote the following from Stöhr, 00, "The nuclei of many gland cells also exhibit varying appearances corresponding to the changing functional state; in empty cells the nucleus exhibits a delicate chromatic network and a conspicuous nucleolus, while in the loaded cells the nucleolus is invisible and the chromatin-cords appear in the form of coarse fragments." Garnier, 00, likewise holds that the most constant correlation between the structure of the nucleus and cytoplasm during glandular activity is the augmentation of chromatin.

The peculiar condensation of chromatin, being more accentuated on the side of the nucleus which is applied to the fibrillated surface, suggests that a condition is thus brought about which is more favorable for the correlation of nuclear and cytoplasmic activities. While I know of no observations which are of precisely the same nature, there are many which show that there is a marked increase in the contact surfaces of cytoplasm and nucleus during periods of great cytoplasmic activity.

Korschelt, 89, has pointed out a number of instances in the eggs of insects where similar changes have been observed. The most striking of these is found in the secreting nurse-cells attached to the eggs of Forficula. Korschelt states that the peripheral position of the nucleus and its richness in chromatin are undoubtedly correlated with cell-metabolism. Carlier, 99, states that in the gland cells of the newt's stom-

ach the chromatin spreads itself out on the inner surface of the nuclear membrane and that this condensation is directly connected with the formation of prozymogen.

These and other facts lead to the conclusion that the position of the nucleus, its greatly increased amount of chromatin and the lateral condensation of the latter are directly related to the formation of fibrillæ.

D. Significance of Correlation of Cytoplasmic and Nuclear Changes.

If it be true that nuclear changes in the muscle cell are correlated with phases of cytoplasmic activity, especially the formation of fibrillæ, we are naturally led to a further inquiry, namely: Does the nucleus of the muscle cell, like that of the gland cell, build up and give off chromatic material, which plays an important part in, if it does not direct, cytoplasmic metabolism?

There are two structures in the muscle fibril which are basophilic in staining reaction, viz., the anisotropic band and the so-called Dobie's line.

Micro-chemical tests made by Macallum, **95**, 219, and Rutherford, **97**, 319, show that this band contains an iron and phosphorus-holding nuclein. Macallum states that in the cells undergoing transformation into striated fibres, some of the chromatin dissolved in the cytoplasm (from the yolk granules) finds its way into the nuclei, as in other cells generally, but the greater part appears to remain in the cytoplasm of the developing fibre, where it later passes into the dark band of the fibril. It is especially noteworthy that Macallum regards this process as exceptional. In general, the chromatin derived from the yolk granules is converted into nuclear chromatin.

While I would in no way question the accuracy of Macallum's work, I think his interpretation that the chromatin in the anisotropic or dark band of the fibril is derived directly from the yolk instead of the nucleus is *a priori* improbable. A serious, if not insurmountable, objection to Macallum's view is the fact that in the regeneration of the adult muscle cell the basophilic portions are differentiated in the entire absence of yolk material. The supposition that chromatin is elaborated in the nuclei for this purpose is confirmed by the fact that before the muscle cell regenerates, the quantity of chromatin is greatly increased through repeated nuclear divisions in the injured end of the muscle cell. These facts have led me to consider the nuclei as the source of the chromatin found in the dark band and Dobie's line, that is, in the basophilic por-

tions of the fibril. If this interpretation be correct, we should find in the muscle cell a process quite analogous to that found in many, indeed most, cells of the organism—the building up and giving off of nuclear material. This material may participate in the elaboration of the various secretions, as is known to be the case in a great many gland cells, or may give rise to more permanent products, such as the hæmoglobin of the red blood cell (Necturus and Amblystoma), the yolk nuclei of the egg, the Nissl bodies in the nerve cell, and, as I believe, the basophile portions of the muscle fibrils.

IV. Conclusion.

The chief results of my studies can be stated in a few words. They show that in the muscle cell of Necturus nuclear differentiation is scarcely less marked than cytoplasmic. They warrant the assumption that nuclear material plays a most important part in cytoplasmic syntheses. They suggest that cellular degeneration and regeneration are accompanied by volumetric, structural and chemical changes in chromatin. Above all, they emphasize the importance of a more precise study of nuclear changes during cytomorphosis.

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LIST OF ABBREVIATIONS FOR ALL FIGURES.

a., apex of cone of fibrillæ.
cm., cell membrane.
c. p., cutis plate.
c. s., cytoplasmic strands.
cy., cytoplasm.
c. z., clear zone.
en., endomysium.
f., fibrillæ.
f. c., fibrillar cone.
ky., karyosome.
l., linin network.
mes., mesenchymal nuclei.
m. p., muscle plate.
n., nucleus.
pl., plasmosome.
s., septa.
y., yolk granules.

EXPLANATION OF PLATE I.

All figures were outlined by the aid of the camera lucida.

Fig. 1.—Represents an oblique longitudinal section through the third myotome of a 6 *mm. embryo*, showing the relation of cutis plate and muscle plate, the accelerated absorption of yolk granules in each end of the myotome and the striations of the myoblast. × 100.

Fig. 2.—Oblique longitudinal section of a *9 mm. embryo*, showing same points as above section, together with the clear zones at either end of the myotome. × 100.

Fig. 3.—Oblique longitudinal section of myotome of *12 mm. embryo*, showing increased extent of fibrillation, and invasion of mesenchymal cells to form septa and endomysium. × 100.

Fig. 4.—End of isolated myoblast from *8 mm. embryo*, after osmic acid fixation, showing cone of striæ or fibrillæ.

Fig. 5.—From freshly teased preparation, showing cytoplasmic strand connecting the ends of myoblasts in adjoining myotomes. × 700.

Fig. 6.—Group of myoblasts from *15 mm. embryo*, showing the septa, endomysium, also the extent of fibrillation in the myoblasts. × 700.

Fig. 7.—Group of contracted myoblasts from *26 mm. embryo*, showing the growing septa, endomysium, peripheral position of nuclei and rows of yolk granules. × 100.

Fig. 8.—Isolated myoblast from *10 mm. embryo*, after osmic acid fixation and safranin, showing arrangement of fibrillæ in the myoblast. × 700.

Fig. 9.—Transvérse section of a group of myoblasts, after Kolossow's osmic acid method, showing the relation of the fibrillæ to the cytoplasmic reticulum. × 700.

Fig. 10.—Transverse sections of myoblasts in *9 mm. embryo*, indicating position of nuclei with reference to fibrillated area. × 500.

Fig. 11.—Transverse section of myoblasts of *15 mm. embryo*, showing variations in mode of extension of fibrillæ and the position of the nuclei. × 500.

FIG. 12.—Transverse section of myoblasts from *26 mm. embryo*, showing the complete fibrillation of the myoblast and the eccentric position of the nuclei. × 500.

FIG. 13.—Transverse section of muscle fibre from *23 cm. adult*, showing the enormous increase in the number of fibrillæ and the redistribution of the nuclei. × 500.

EXPLANATION OF PLATES II, III, IV.

In making the drawings of the various nuclei the writer used a Zeiss apochromatic 2.0-mm., homogeneous immersion lens in combination with a No. 12 compensating ocular. The drawings were made at the table level under camera lucida projection. The greatest care has been taken to reproduce faithfully the appearances.

The nuclei were all fixed in Flemming's stronger solution and then stained by Heidenhain's iron alum hematoxylin method or with safranin. It should be added that these stains were supplemented by a number of others, all of which give the same appearances.

FIG. 14 is a profile drawing of an isolated nucleus, in the resting stage, from a myoblast of the *7 mm. embryo*.

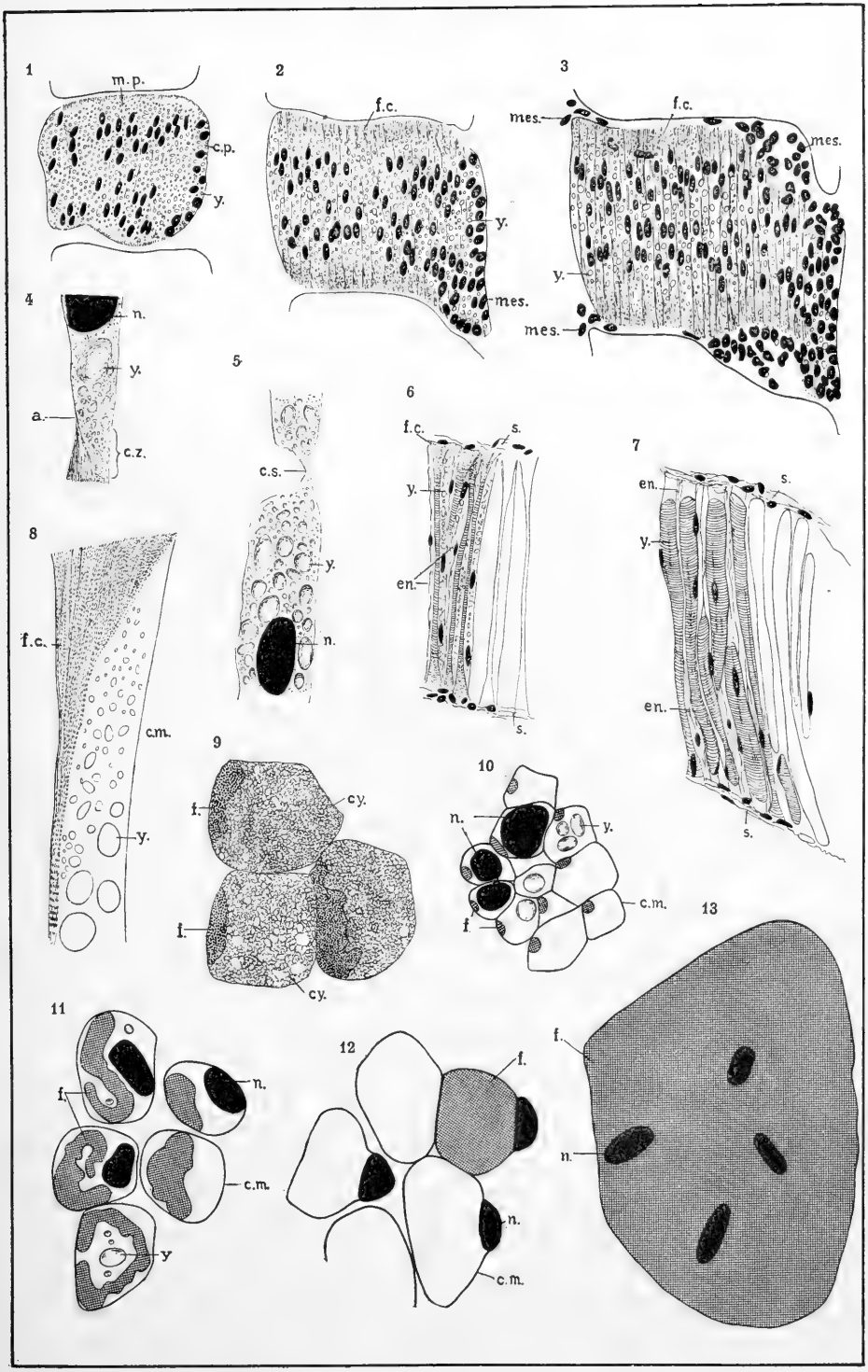
FIGS. 15-26 represent a series of transverse sections of a nucleus in the same stage.

FIG. 27 represents a drawing of an isolated nucleus, in the resting stage, from a myoblast of a *26 mm. embryo*.

FIGS. 28-39 represent a series of transverse sections of a nucleus in the same stage.

FIG. 41, profile view of an isolated nucleus, in the resting stage, from muscle cell of *23 cm. adult*.

FIGS. 42-60 represent a series of transverse sections of a nucleus in the same stage.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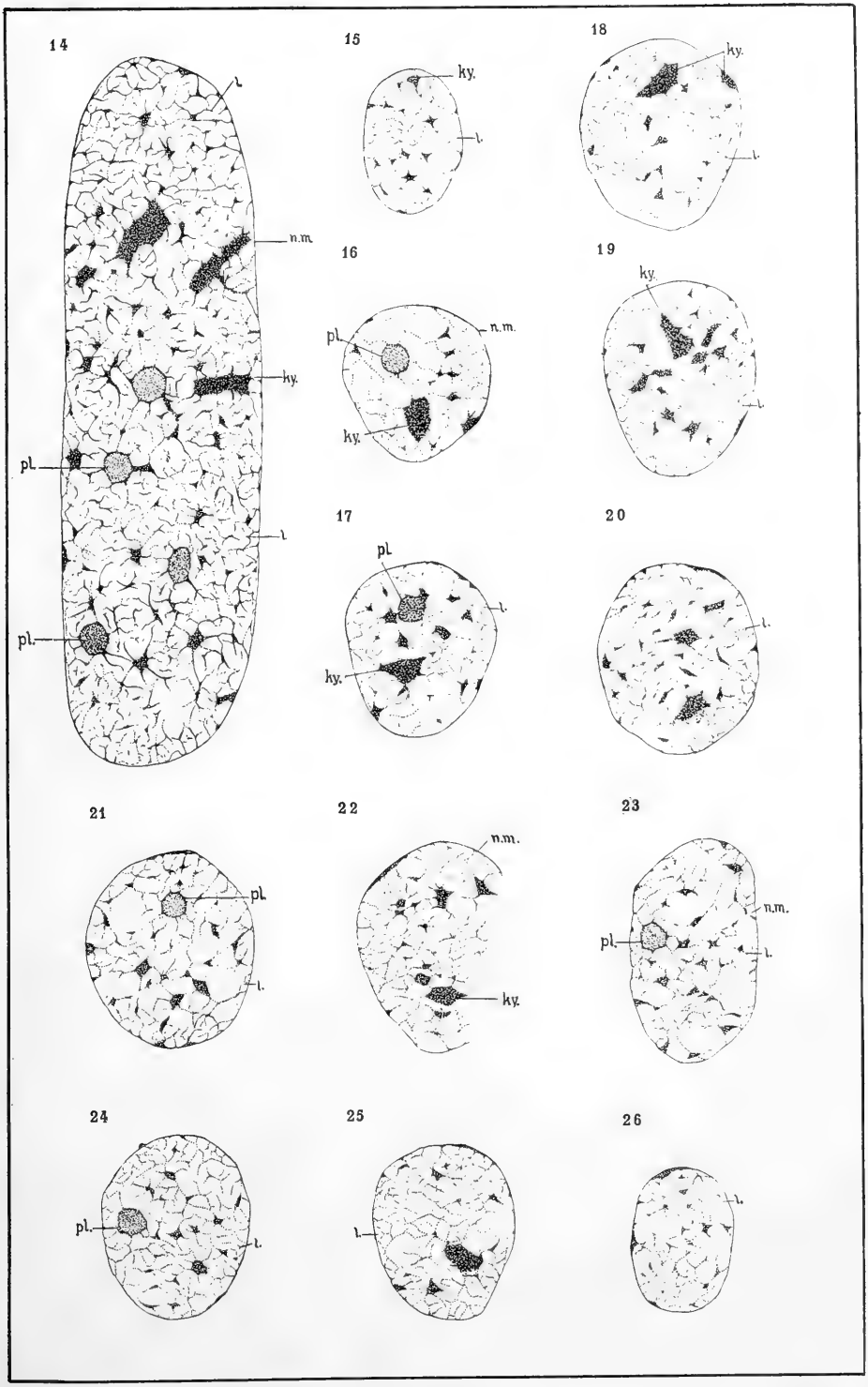
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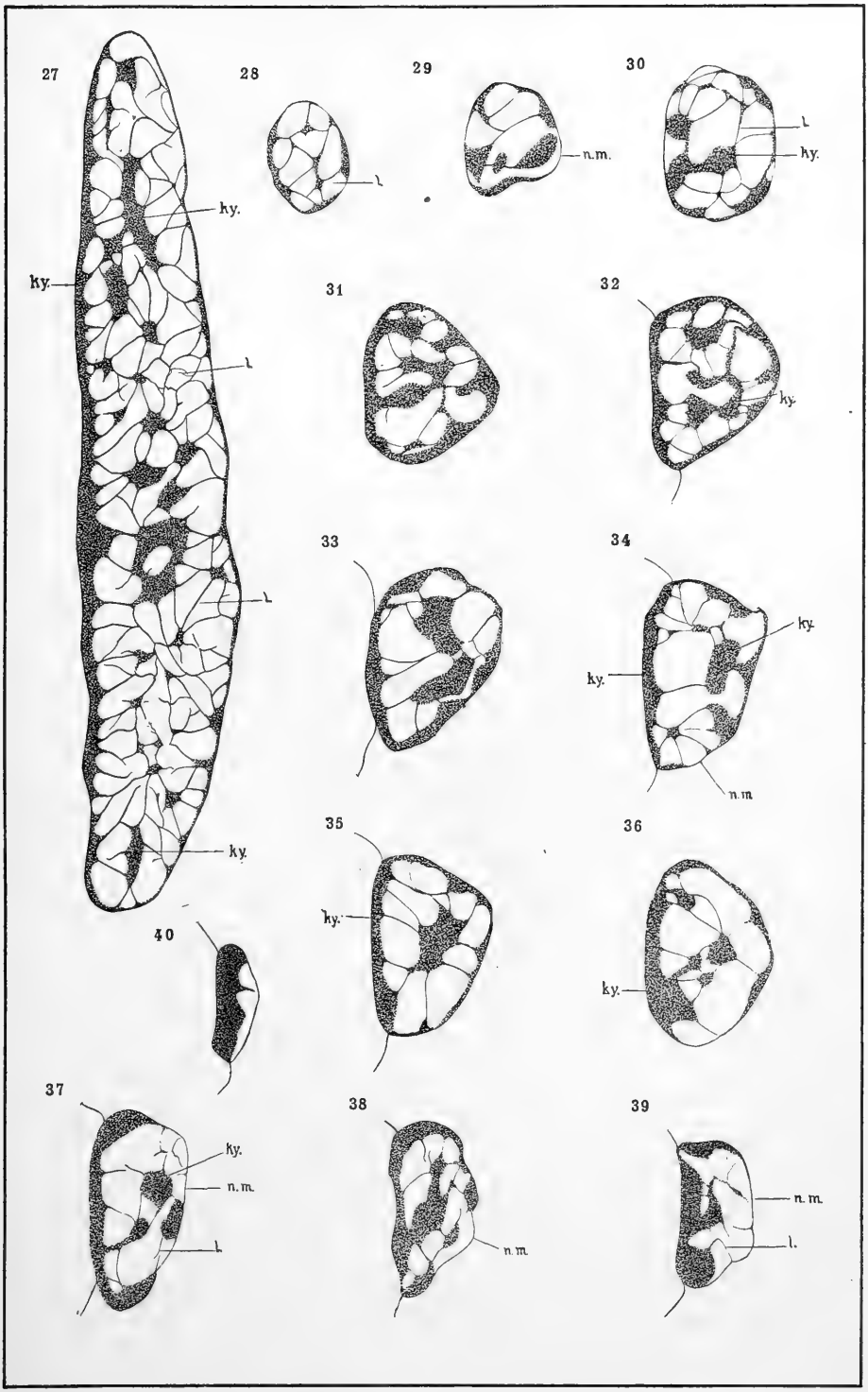
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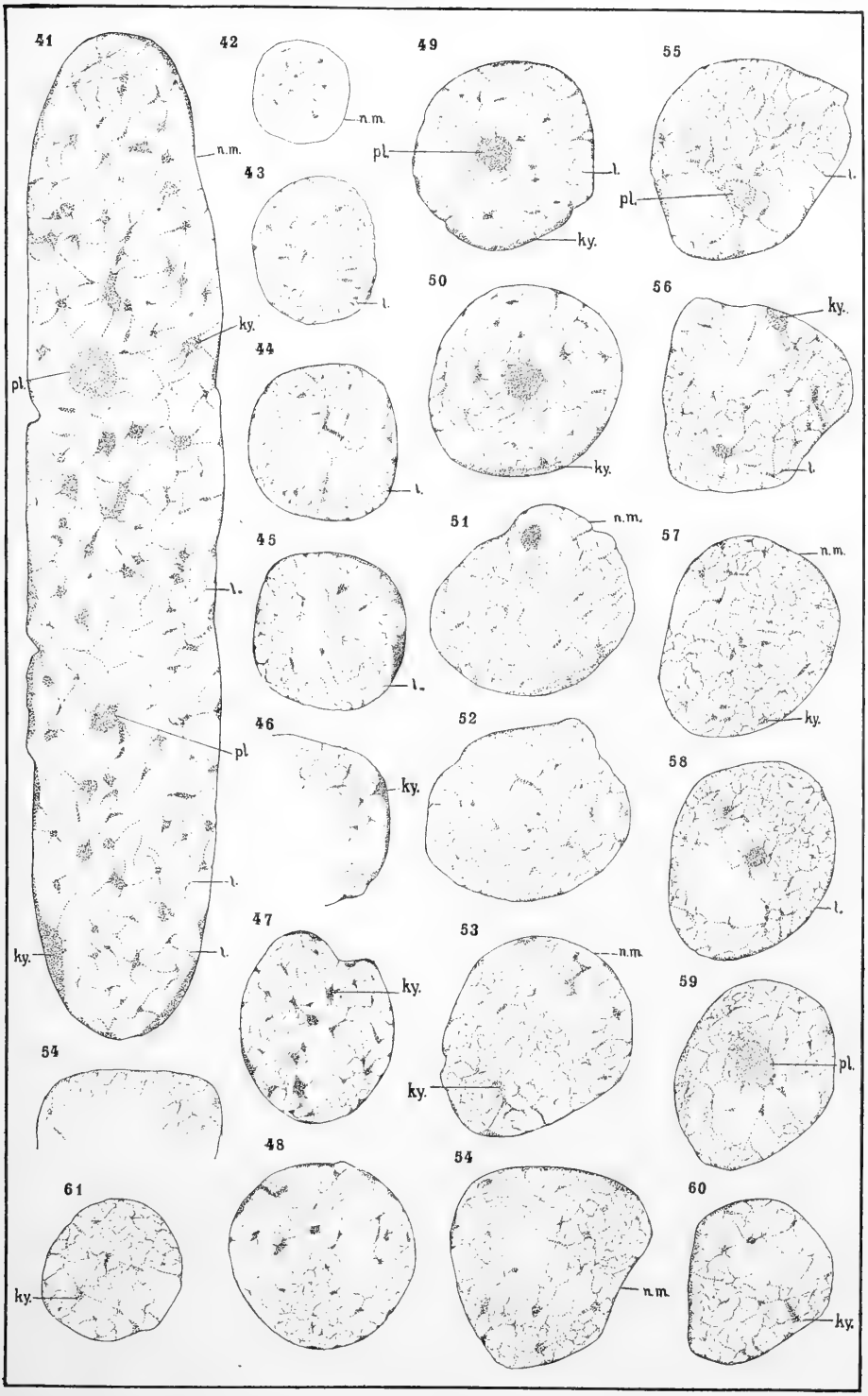
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RESEARCHES ON THE OOGENESIS OF THE TORTOISE, *CLEMMYS MARMORATA*.

BY

JOHN P. MUNSON.

WITH 7 PLATES.

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Cytological problems are so numerous and yet so prominent in the minds of investigators that it may seem unnecessary to call attention to them again here. Oscar Hertwig, E. B. Wilson, Whitman and others have stated these problems so well that I cannot do better than to refer to these writers, and call attention to the following suggestive quotation from Wilson, **84**: "On the one hand, it has been suggested by Flemming and Van Beneden, and urged especially by Whitman, that the cytoplasm of the ovum possesses a definite primordial organization, which exists from the beginning of its existence even though invisible, and is revealed to observation through polar differentiation, bilateral symmetry, and other obvious characters in the unsegmented egg. On the other hand, it has been maintained by Pflüger, Mark, Oscar Hertwig, Driesch,

Watase, and the writer (Wilson) that all the promorphological features of the ovum are of secondary origin; that the egg-cytoplasm is at the beginning isotropous, *i. e.*, indifferent or homaxial, and gradually acquires its promorphological features during its preembryonic history.[1]

In the work of the writers cited above, the literature on the subject up to the present time is extensively reviewed, especially by Wilson, **84.** I may perhaps be justified, therefore, in confining myself in the present paper to a concise statement of my own observations. To show their relation to the work of other observers, and the bearing of my conclusions on present-day theories, would make my paper undesirably lengthy and involved. I hope in the near future to consider this phase of the subject in connection with observations that I have made on the ovarian egg of the crayfish.

My present observations have been made on the ovarian egg of the Tortoise—*Clemmys marmorata.* I have found it a favorable egg to work with, and have been gratified to find so many of my conclusions regarding the history and organization of the egg of Limulus, beautifully confirmed.

Regarding my paper on that subject, I take pleasure in expressing here my appreciation of the favorable mention which it has received; and I desire especially to express my thanks to Prof. C. F. Hodge of Clark University, to Prof. Dr. R. Fick of Leipsic, and to Dr. Fritjof Nansen of Christiania for very kind courtesies and favors.

DESCRIPTION OF THE TORTOISE.

Clemmys marmorata is a tortoise inhabiting the western part of North America. I have had no opportunity to study its distribution. But as I am not aware that it exists east of the Rocky Mountains, and as it is not mentioned in Jordan's Manual of the Vertebrates, I assume that it is not common. It inhabits the ditches, pools and ponds tributary to the Yakima River in central Washington. My identification is based on three specimens found in the Museum of Natural History at Victoria, British Columbia. The following description may not be out of place here:

Carapace ovate, in the adult considerably elongate; margin flaring, not strongly convex; highest in the middle; length from head to end of tail, ten inches; plastron of twelve plates covering the whole undersurface; lobes not hinged; alveolar surface of jaws medium in width; alveolar groove visible; upper jaw slightly notched in front; carapace

[1] The Cell in Development and Inheritance.

depressed, not keeled; toes strong, broadly webbed; carapace dark green; plates of carapace in quincunx, and margined with paler brown below; concentric striation of cestal plates visible but not strongly marked; marginal plates not united in the adult, apparently so in the young; adjacent edges of posterior marginal plates forming a compound curve; marginal plates slightly notched in front; marginal plates twelve, with two narrow supernumerary in front; anterior and posterior marginal plates divided by vertical, yellowish stripe; lateral plates with slightly reticulate, yellowish markings. Head small, hind legs clubshaped, larger than forelegs; four toes of hind feet with long claws, five claws in front. Marginal plates ornamented below with conspicuous, bright red lines; feet and tail black, striped with yellow; head and neck green, covered with smooth skin; side of head and neck marked with yellow stripes converging in front of the eye, and crossing the iris. Plastron red or pink, and marked with a bilaterally symmetrical design of brown, which is very characteristic both in the younger and in the older forms.

Methods.—Preserving fluid, picro-nitric; dehydrated in 15-100 per cent alcohol, passed through chloroform and imbedded in paraffine; sectioned five μ, and stained on the slide. The following stains have been used, and have been found useful about in the order named: saffranin, acid fuchsin, Delafield's hæmatoxylin, picro-carmine, eosin, borax carmine, ammonia carmine, orange G., Bismark brown, Vesuvin brown, violet blue, dahlia violet, iodine green, Congo red, anilin blue, anilin red. Many of these stains were also variously combined. Thus: Hæmatoxylin and picric acid; hæmatoxylin and eosin or acid fuchsin, hæmatoxylin and saffranin, etc. I have also found it profitable to study the egg in the living state or merely hardened and killed without imbedding or sectioning. Iodine applied to eggs in this condition under the cover-glass gives valuable and interesting results. It is surprising that iodine, which has been found so valuable in the study of living plant tissue, has not been more extensively used in the cytology of animals.

THE OVARY.

On removing the plastron of the adult animal, at the proper season of the year, after the first of May, the ovary, with its numerous large, yellow eggs, is the most conspicuous internal organ exposed. It lies in the abdominal cavity, and when the eggs are grown, or nearly so, fills the abdominal cavity between the hip girdle and the shoulder girdle. One such ovary contains, besides fifteen or twenty comparatively large spherical eggs, measuring three-fourths of an inch or so in diameter, and having a deep yellow coloration, many stages of the growing eggs down to

the very earliest, including the oogonia. The smaller eggs are paler in color, and they are distributed irregularly between the larger eggs, as seen in Plate VII, Fig. 92.

The ovary is covered with a thin membrane, evidently a fold of the peritoneal membrane, and each egg is surrounded by two distinct coats of membranous tissue, which are developed from the stroma of the germinal mass. These latter membranes are richly supplied with blood-vessels.

The Germinal Mass.

I have not traced the origin of the germinal layer in the embryo. From the matured ovary, I infer that it develops in connection with the peritoneal lining of the abdominal cavity, the original germ-cells becoming surrounded by thin membranes apparently continuous with that lining membrane.

The germ-cells form a mass rather than an epithelium; and, in the adult ovary, are divided up into distinct masses having more or less the form of flattened oval ridges, slightly longer than broad, and distributed between the larger eggs. It may be that this separation into ridges is due to the growth of the eggs; and that, in the very young ovary, it forms one continuous mass.

The position of the smallest eggs in Plate VII, Fig. 92, indicates the general arrangement of the germinal ridges, one ridge being usually associated with each of the smaller eggs. There is represented in Plate I, Fig. 1, a longitudinal section of such a germinal ridge; Plate I, Fig. 2, a transverse section; and Plate I, Fig. 3, a horizontal section of a germinal ridge.

The Oogonia.

The germinal ridges consist chiefly of spherical cells, the oogonia, each one being surrounded by a layer of cells, forming the stroma of the ovary. Each of these stroma cells has a central flattened nucleus, staining deeply, and all forming a circle around each oogonium, their arrangement is such as to suggest a follicle; but the elongated and flattened shape of these nuclei, as well as their closely packed chromatin and consequently deep staining, renders them easily distinguishable from the true follicle, which forms later within. It is this layer which evidently forms the innermost tunic immediately surrounding the follicle epithelium of the growing egg.

The oogonia are spherical or slightly elongated. The nucleus is large and spherical, and shows, at first, a very distinct network, apparently imbedded in the hyaline karyolymph. The oogonia vary in size. In

the larger ones, the hyaline karyolymph becomes turbid by the deposit of chromophilous granules. This renders the nucleus more conspicuous, owing to its increased size, and its greater staining capacity. The cytoplasm of these oogonia is rather hyaline, and does not stain at all deeply in nuclear stains. The cytoreticulum can, however, be seen.

The Centrosome.

In the immediate neighborhood of the nucleus, in the cytoplasm, there is a true centrosome. The amount of cytoplasm is not great, but at one pole of the nucleus there is more of it than elsewhere. The centrosome can here be seen in the form of a tiny body close to the nucleus. It may be more or less conspicuous according to the amount of archoplasm surrounding it. The archoplasm is apparently not always present. The centrosome itself, *i. e.,* the central granule, is an exceedingly minute body which comes into view only on focusing, when it often stands out sharp and clear in the center of what seems to be a clear globule, surrounded by a ring of microsomes. In fact, I have seen this clear globule often when I have been unable to make out the central granule—a fact that may be due to defective focusing. From the circle of microsomes surrounding the clear globule, the cytoreticulum radiates, usually becoming crowded along the sides of the nucleus and forming a thin layer investing the nucleus. My experience with these germ-cells has made me very suspicious about all negative evidence concerning the centrosome. That such a tiny granule is not always visible is not strange, when it is noticed how the cytoplasm varies as regards its density and transparency, not only in variously preserved material, but in the living state as well. As is plain from the later history of the centrosome, to be related presently, the presence or absence of the central granule may or may not be important. What is of greater importance, perhaps, than the central granule is the circle of microsomes surrounding it, and from which the fibrils of the sphere seem to radiate. To say that this central granule is homologous to or identical with the ordinary cytomicrosome does not signify much either one way or another, so long as the radial system of fibrils in its immediate vicinity can be shown not only to exist at this early stage, but to persist throughout subsequent stages of the growing egg. The extreme tenuity, also, of the radial fibers of the sphere, especially in the resting condition of the astral system, often makes it seem more surprising that they can be seen at all than that they should at times become obscured by granules or otherwise become invisible, be it due to reagents used in preparing the tissue or to varying states of the fibers. The fact seems to be that the fibers of the cytoreticulum have the power of

contraction by which the microsomes are made to approach one another. The circle of microsomes may thus become closely applied to the central granule, giving rise in that way to the conspicuous centrosome which appears when the oogonium divides.

Division of Oogonia.

The marked difference in size of the oogonia must mean that they grow considerably before dividing to form oocytes. This growth consists in a marked increase in size of the nucleus and the greater amount of chromatin or at least chromophilous granules, as well as in a marked increase in the amount of cytoplasm. During this growth the radial zone of the sphere persists (Plate I, Figs. 1, 2, 3).

The division of the oogonia is mitotic (Plate I, Fig. 3). The chromatin becomes massed into a spireme, a spindle is formed with a centrosome at each pole of the spindle. I have no observations on the division of the centrosome to record. But I presume that the two centrosomes result from division of the original centrosome. This fact may be noted, however, that the centrosomes are now very much more conspicuous than the original centrosome, due, as it seems to me, to the tension of the astral system, the massing of the archoplasm around the centrosome and the concentration of the circle of microsomes so as to make them seem merged into the central granule.

When an oogonium enters the division period, it passes through a series of divisions with a very brief period, if any, between each division (Plate I, Figs. 2, 3). Thus it first divides into two (Plate I, Fig. 6). The two cells thus formed (Plate I, Figs. 8, 9) divide again immediately after the reconstruction of the nuclei, giving rise to four similar cells (Plate I, Fig. 9). After a brief interval, these again divide, giving rise to eight daughter-cells, from the original oogonium (Plate I, Fig. 10). As might be expected, the eight cells thus formed are very small as compared with the original oogonium (Plate I, Fig. 3). As can be seen in Plate I, Fig. 3, not only are the spindles associated into groups, but the progeny of each oogonium lies crowded together in nests of two, four or eight cells, and are surrounded, as was the mother-cell, with the inner layer of the stroma cells.

Formation of Follicle.

One of the eight cells, resulting from the repeated division of the oogonium, becomes the oocyte or egg; the rest become the follicle. The follicle cells are, therefore, the sister-cells of the egg. The oocyte is

always the central cell. It differs from the follicle cells, so far as I can see, in two important particulars, namely: First, its central position gives it an environment of similar cells; while the follicle cells have one side adjacent to the egg, the other side adjacent to the surrounding stroma cells. One would naturally expect that, if the differences in surroundings could develop polarity, such should be found in the follicle cells, while the egg should be homaxial. On the contrary, the oocyte differs, secondly, from the follicle cells in having a centrosome at one pole, which is evidently absent in the follicle cells.

Differentiation of the Oocyte.

Seeing that the oocyte resulting from the division of the oogonia is always the central cell, I have endeavored to ascertain the probable cause of this. Is the egg a result of its accidental position amid its sister-cells, and do the follicle cells simply become follicle cells because of their accidental position with reference to the oocyte on the one hand and the surrounding stroma cells on the other? In other words, is it a matter of chance which of these cells shall become an egg, or is there some internal difference in the cells, which results from a qualitative division of the original oogonium? Right here, it seems to me, lies the problem of all problems, that of cell differentiation. The matter presents itself here in its simplest form; for we have here evidently to do with the first of those changes, ecdysis or moults through which the original germ separates off from itself the somatic cells, which nourish and protect it, and of which the development of the fertilized egg is only a more complex process. May not this division of the oogonia be compared to a simple process of cleavage, by which there results the most primitive separation into germ and somatic cells? If intrinsic differences arise in this group of cells from a qualitative division of some sort, it ought to afford a strong presumption in favor of such a process in the development of the fertilized egg; if, on the other hand, the difference between the oocyte and the follicle cells is due to cellular interaction, may this factor not be equally important in the later ontogeny?

I have endeavored to discover the law according to which one of the cells of this group comes to occupy a central position, but I cannot say that I have been successful. There appears to be no regularity in the direction of the spindles in the division of the four cells into eight, which might determine the final position of the central cell. I am not prepared to say, however, that no such law exists. Possibly the following facts are sufficiently important, in this connection, to warrant a statement of them.

At the close of the last division, the cells are arranged as seen in Plate I, Figs. 10, 11. The chromosomes evidently become vacuolated, by the secretion of a hyaline matrix, which separates the chromatin substance into granules arranged on delicate fibres of linin, forming a network. At first, all the nuclei thus formed are about equal in size, all being spherical, and having the chromatin more abundant around the periphery. The cytoplasm is relatively scarce, forming only a thin layer around the nucleus of each cell. Comparing Figs. 10 and 11, it can be seen that all the cells just divided, and before the nuclear reticulum is fully formed, are very similar, each having a centrosome surrounded by archoplasms, situated at one pole of the nucleus. In Plate I, Fig. 11, on the other hand, only the central cell has an undoubted centrosome at one pole. Even in this cell, the centrosome is not so distinct as previously, the archoplasm having apparently spread out along the sides of the nucleus, forming a crescent. The centrosome is now a tiny granule, occuping a clear globule, which is surrounded by a circle of larger microsomes. Evidently this is the resting condition of the sphere, the fibrils being relaxed, and the microsomes of the peripheral ring separated from the central granule, rendering the whole slightly more difficult to see. In the peripheral cells, on the other hand, the slight quantity of cytoplasm surrounds the nucleus equally on all sides. But the nucleus in these future follicle cells show, even in this early stage, a comparatively large central body which resembles a nucleolus. It is difficult to believe, however, that it is a nucleolus, since the true nucleoli develop much later. I am inclined to believe that this is the centrosome of the preceding stage. These cells, so far as can be seen, have a radial symmetry, possibly due to the position of the centrosome within the nucleus. The central cell, the oocyte, on the other hand, shows the centrosome in the cytoplasm at one pole, and hence has a more oval form. A distinct polarity, in other words, exists here; and this seems to be due to the relative position of the nucleus and centrosome respectively. I can see no reason whatever, for doubting that this centrosome is the centrosome of the dividing oogonia (Plate I, Fig. 11), and that the transition from the condition existing in Fig. 10 to that of Fig. 11 is a complete transformation, and a formation *de novo* of the centrosome in the central cell. This centrosome is not a transient body, as the subsequent history of the growing oocyte shows. This fact, too, can hardly be denied, namely: That, first, the nucleus of the central cell or oocyte, now the germinal vesicle, in its earliest stage, is derived directly from the chromatin of the dividing oogonia, and hence is a direct continuation of the nucleus of the oogonia; and, second, that the cytoplasm, instead of being formed *de novo*

from the young germinal vesicle, is also a direct continuation of the cytoplasm of the original oogonia. The cytoplasm being so limited, it is easy to regard the sphere as the most essential part of the cytoplasm, and from the later history of this body in the growing egg, one is almost tempted to infer that a chromosome organically connected with a centrosome and sphere is sufficient to develop a nucleus from the former and cytoplasm from the latter, *i. e.*, a cell, in the present case, the egg. In this early stage, immediately following the reconstruction of the nucleus, (now the germinal vesicle), from the chromosomes of the spindle, it is hardly possible that metabolic processes in the nucleus could be responsible, on the one hand, for the central nucleolus-like body in the nucleus of the peripheral (follicle) cells; or, on the other hand, for the accumulation, at one pole of the young germinal vesicle, of the slightly granular centrosome and sphere.

The staining reaction of the chromatin and archoplasm respectively is so different that the origin of the one from the other could not even be suggested by it. The chromatin stains deeply and easily in nuclear stains, the centrosome and archoplasm, on the contrary, are conspicuous chiefly for their resistance to nuclear stains.

I conclude from the above facts that there are important internal differences between the follicle cells and the oocyte at this earliest stage. The principal difference is the position of the centrosome in the oocyte, which not only gives it a polarity, but also seems to confer on the oocyte the capacity for growth. It is this centrosome and sphere which later grows so extensively by the absorption of food and the formation of yolk in the later stages as can be seen by examining Plate VII. The probable function of the nucleus in this later growth is suggested by the origin and history of the yolk-nucleus to be described later on in this paper.

THE EGG.

Stages of Growth.—The history of the growing oocyte presents three successive phases, which may be used as landmarks for descriptive purposes.

The first period extends from the beginning of growth, to the time when the cytoplasm assumes its characteristic granular appearance; at which time, also, the true nucleoli make their appearance in the germinal vesicle.

The second period extends from the first period to the beginning of true yolk-formation; and the *third period* covers that period of growth in which the true yolk-bodies are formed.

Stage I.

The Follicle.—From the very first, the appearance of the oocyte, differs from the follicle cells in that the chromatin of the latter, at first very similar to that of the young germinal vesicle, being in the form of distinct network of irregular granules suspended in a clear nuclear matrix, increases considerably and consequently stains more deeply. As the oocyte grows, the nuclei of the follicle cells lose their spherical form, and become more or less flattened, the elongation being in a plane vertical to the egg surface.

The young germinal vesicle preserves its spherical form. It seems to grow rapidly—much more so, at this time, at least, than does the cytoplasm. At first, the ground substance of the germinal vesicle is clear, showing the chromatin network beautifully. The increase in size seems to be due to the increased amount of karyolymph. At first the chromatin has the form of granules suspended in or attached to a network of hyaline threads, but this lasts only for a brief period. The granules increase rapidly and soon obscure the hyaline matrix and the nuclear network. Consequently the young germinal vesicle stains more deeply now.

At first some of the granules imbedded in the linin network are larger than the rest; are more spherical; stain more deeply, and are distributed about equally throughout the germinal vesicle (Plate VII, Fig. 91; Plate I, Fig. 17). These spherical bodies become obscured as the irregular granulation of the matrix becomes more marked. I suspect that it is these larger spherical chromatin bodies that are more or less directly responsible for the granules appearing around them.

The Nucleoli.—The whole germinal vesicle being filled with granules, till only traces of the original network can be seen, there appears at the periphery one or two bodies larger than the former spherical chromatin bodies, and having all the characteristics of true nucleoli. The principal characteristics which serve to identify this as a nucleolus are: First, its position, which is identical with that of all the subsequent nucleoli which make their appearance; and second, the appearance, within it, of vacuoles, which cannot be seen in the spherical bodies of the network. As regards size and shape, it is not especially distinguishable from the larger spheres of the chromatin network. In its staining reaction, also, it resembles those bodies. On account of its peripheral position, however, I entertain considerable doubt as to its being one of those early spheres merely augmented in size.

The cytoplasm, also, at first becomes more and more turbid, and

increases in amount. The granules causing this turbidity are at first very minute, and do not stain so intensely in nuclear stains as the smaller granules of the germinal vesicle. The cytoreticulum is, however, made evident by such stains as eosin and picro-carmine, and even by hæmatoxylin.

The centrosome retains more or less completely the characteristics which it possesses just after the telophase of karyokinesis of the oogonium. It is not always possible to see the tiny central granule. The circle of large microsomes is more easily seen. It encloses a clear, glassy, round opening or globule (Plate I, Fig. 14). The archoplasm surrounding this usually extends to the germinal vesicle, partly enclosing it, thus forming a crescent-shaped granular area, in the widest portion of which the centrosome and sphere can be seen. The granular archoplasm sometimes obscures the centrosome structure either partly or completely, in which case only an irregular mass of granules marks the location of the centrosome. Occasionally, too, the archoplasm flows around the germinal vesicle, forming a ring (Plate I, Fig. 15), at one pole of which the centrosome and sphere are to be seen. This consists of a small central granule, from which radiate tiny fibers in all directions to comparatively large microsomes which, owing to their size, form a dark ring around a light area immediately surrounding the central granule, and across which the slender radiating fibrils extend. From this first ring of large microsomes, there extend similar radiating fibers to a second ring of microsomes slightly smaller than the first and situated about half way between the inner ring and the periphery of the egg (Plate I, Fig. 17). The entire contents of this second ring stain more deeply than the rest of the cytoplasm, but not nearly so intensely as the germinal vesicle. It is in close contact with the germinal vesicle and is indented at the point of contact, so that it, together with the spherical germinal vesicle, forms an oval area in the center of the young egg, surrounded by a layer of less granular protoplasm of about equal thickness (Plate VII, Fig. 97, *p. z.*).

Cytoplasmic Areas.—As this outer protoplasmic layer is distinguishable throughout the later history of the egg, and must be referred to frequently, I deem it best to give it a name, and shall call it the *peripheral zone* (Plate VII, Figs. 97 and 88, *p. z.*). The outer portion of this zone is further differentiated into a thin layer immediately under the egg-membrane. I shall call this the subcuticular layer (Plate VII, Fig. 85, *s. c. l.*).

The line separating the peripheral zone from the germinal vesicle and sphere, taken as a whole, I shall call the cytocœl (Plate VII, Fig. 97,

cy. c.); and the sphere itself, because of its many peculiarities, not usually recognized as belonging to the centrosome and sphere, I shall call the *cytocenter* (Plate VII, Fig. 97, *c. c.*, Fig. 85, *c. c.*). I take this cytocenter, in the larger eggs, to be the typical centrosome and sphere of the earlier stages, modified by growth and by the deposit of yolk-bodies and yolk-granules.

I am very reluctant to introduce these names into an already overburdened vocabulary, but see no way of expressing myself without them.

I have said that there are two rings of microsomes surrounding the centrosome, forming the structural basis of the true attraction sphere (Plate I, Figs. 12, 13, 14, 15, 16, 17). That must be true in the very early stages of development. In Fig. 17 is represented a young growing egg more highly magnified. In Plate I, Fig. 13, is represented a section through the attraction sphere at right angles to the egg-axis. But the same appears to be true, also, of the oogonia (Plate I, Figs. 1, 2, 3, 4, 5, and Plate VII, Figs. 93, 94, 95). The number of these circles seems to increase as the egg grows (Plate VII, Figs. 96, 97; Plate II, Figs. 38, 50, 51; Plate III, Fig. 55).

Stage II.

The nucleolus, having first made its appearance in the preceding stage, the number of these now increases rapidly. They correspond roughly with the size of the germinal vesicle, increasing in number as it grows. From a single nucleolus at the beginning, there may be a hundred or more in the fully-grown germinal vesicle—a fact which has led me to doubt their direct descent from the chromatin spheres of the first stage. They vary considerably in the fully-grown germinal vessicle of the third stage (Plate VII, Figs. 86, 87; Plate II, Figs. 30, 43, 63). Their staining reaction is similar to that of chromatin. Hæmatoxylin and borax carmine make them conspicuous. The larger ones usually show the central differentiation or vacuole common to most nucleoli. It is rare, however, that they possess more than one of these (Plate III, Fig. 63, 64, 65).

The germinal vesicle in this egg presents a somewhat remarkable uniformity as regards form. It is spherical, at times slightly oval, and seems to retain this form from its beginning (Plate I, Figs. 11, 12, 18, 19; Plate II, Figs. 43, 44; Plate III, Fig. 62; Plate VI, Fig. 86?), and even late into the final period of growth when the egg becomes filled with yolk (Plate VI, Figs. 85, 86).

Evidence of the nuclear reticulum is present throughout the three stages, though the granular karyolymph renders the network indistinct,

especially in cytoplasmic stains, because of the increasing affinity of its granules for such stains. Hæmatoxylin and picro-carmine, however, make certain aspects of the reticulum very evident. The finer strands of the network, so beautifully evident in the early stage, are not now visible; but bead-like rows of deeply-staining spheres, somewhat resembling the smaller nucleoli, appear as isolated or continuous strands running in wavy lines through the granular matrix (Plate III, Fig. 63; Plate IV, Fig. 68; Plate V, Fig. 75; Plate VI, Fig. 82). From the bead-like bodies of which these chromosomes are composed, there seem to radiate delicate fibrils, giving a woolly appearance to the chromosome bands. This is not visible at all times in the same kind of material. I presume it is due to the finer fibrils of the obscured network.

The position of the germinal vesicle, as in the preceding stage, is very constant. It is never exactly at the center of the egg. Its eccentricity seems to be constant, though I cannot say that it is absolutely so. In sections at right angles to the egg-axis, it is central (Plate III, Figs. 57, 58, 59). But in sections parallel with that axis, it is always removed from the center; and, in most if not in all eggs in this and the preceding stage, occupies a position about midway between the egg-center (cytocenter) and the periphery. An inspection of the plates will hardly tend to convince one of the truth of this statement; but in many, if not all cases, the exceptions in this respect are due to the fact that the section does not coincide with the egg-axis, or else does not pass through the center of the germinal vesicle.

The cause of this constant eccentricity of the germinal vesicle is the presence, at the egg-center, of the centrosome and sphere, which in this and in later stages I have called the cytocenter, partly because, although it is a direct continuation of the centrosome of the dividing oogonia, and of the sphere of the earliest stage of the oocyte, it often departs so far from what has generally been understood by the term centrosome and sphere.

The eccentricity of the germinal vesicle is such, that the cytocœl (outer limit of cytocenter) intersects it considerably below the middle (Plate I, Figs. 25, 26; Plate II, Figs. 29, 34, 35, 36, 38, 39, 40, 49, 50, 51). Comparing these figures with Plate I, Figs. 12, 14, 16, 17; Plate VII, Figs. 96, 97, 84, it becomes evident how little this relation has changed even in eggs of the considerable size represented in Plate VII, Figs. 84, 85, 86. I have said that the germinal vesicle is always eccentric. This it must necessarily be so long as the centrosome, and later the cytocenter, occupy the position they do. The cytocenter is always present in this egg, and its persistence throughout this and later stages of the growing

egg should be important evidence of the persistence of the centrosome of which it is a direct continuation.

The cytocenter, notwithstanding its many peculiarities, often presents, even in this second stage of the egg, when the cytoplasm has become very granular, the principal features of a typical sphere, with a central granule or granules, such as we find at the beginning of growth (Plate I, Fig. 22; Plate II, Fig. 38; Plate III, Figs. 56, 65; Plate VII, Figs. 96, 97). Furthermore, it often shows very distinctly the surrounding radiations of the true aster (Plate I, Fig. 23; Plate II, Figs. 30, 33; Plate III, Figs. 56, 60; Plate IV, Fig. 68).

The central granule is not always visible. Its place may be occupied by what seems to be a round hole or an unstained transparent body (Plate II, Fig. 49, 51; Plate III, Fig. 65), or by an irregular network (Plate III, Fig. 62; Plate V, Fig. 75; Plate VI, Figs. 78, 82). This network-condition of the center is most frequent in the third stage of the egg, when the yolk-bodies are being formed at the periphery. The network often has a denser central portion (Plate VI, Figs. 81, 83; Plate IV, Figs. 69, 71), in the center of which a deeply-staining body often appears (Plate IV, Fig. 69; Plate VII, Fig. 84). Radiating from this dense central body, are numerous straight fibers passing through the network out into the cytoplasm of the peripheral zone, suggesting most certainly the original sphere with its radial fibers, etc.

A form of the cytocenter, which is more common in the early stages of the second period of growth, is that of a comparatively homogeneous, slightly granular or fibrous mass, as seen in Plate I, Fig. 25; Plate II, Figs. 27, 29, 45, etc. Slight or even pronounced differentiation of this can in most cases be made out as in Plate IV, Fig. 67; Plate III, Figs. 55, 64. The more homogeneous ones of this kind are possibly caused in part by the reagents, for they are occasionally contracted so as to leave an open space extending partly around them (Plate I, Fig. 25; Plate II, Fig. 29). But this does not appear in those represented in Plate II, Figs. 27, 45.

It is difficult to suggest any reason why the reagent should have such effect in one case and not in others. The cytocenter assumes these different forms in the same ovary, treated with the same reagents. Many of the different forms can be seen on a single slide or on a series of slides made from the same serial sections of a single ovary.

While different stains differ in their power of rendering the fibers and granules prominent, the variety of forms can by no means be attributed to the effect of stains.

Staining Effects.—The cytocenter is eminently cytoplasmic in its stain-

ing reaction. A center, like that represented in Plate IV, Fig. 69, can be differentiated by acid fuchsin following hæmatoxylin so that alone stands out like a bright red astral body, all other parts of the cell retaining the hæmatoxylin stain.

All parts of the germinal vesicle take the hæmatoxylin stain, and retain it after application of acid fuchsin or eosin. The granular matrix of the germinal vesicle has a paler coloration while the nucleoli are most deeply colored by this stain. When hæmatoxylin is followed by picric acid, the granular matrix is strongly affected, while the nucleoli resist its action, as does also the chromatin network, especially the spherical chromosomes (Plate IV, Figs. 67, 68). Hæmatoxylin has very little effect on the cytocenter. Appearances like those represented in Plate II, Figs. 27, 41, are apparently frequent after this stain. When hæmatoxylin is followed by acid fuchsin, the cytocenter is the most conspicuous part of the section.

Forms like those represented in Plate II, Figs. 29, 45; Plate IV, Fig. 66, are made conspicuous by eosin. A cytocenter of an egg about the size of that represented in Plate II, Fig. 49, from a section stained with eosin, is represented in Plate VII, Fig. 90, as it appears under a high power. That it has the essential structure of the original centrosome and sphere of the very youngest eggs, as that represented in Plate I, Fig. 17, for instance, is quite evident. Owing to the great increase of the amorphous granules of the cytolymph, the fundamental structure is obscured. But it can, nevertheless, be seen that it consists, as in the young egg, of a darker center surrounded by a less dark ring; and this, again, surrounded by definitely limited zones, which again are surrounded by a wider zone of open meshes of fibers apparently in the form of a network. Through this outer network of fibers there can also be seen radial fibers proceeding from the inner zones. I have taken special pains not to exaggerate these features in the section. It is hardly necessary to say that an exact reproduction, in pencil drawings, is difficult if not impossible. Yet Plate VII, Fig. 90, is as near a true picture as I can hope to make it. I feel confident that everything represented in the plates can be seen by any unprejudiced eye, from the slides from which the drawings are made. Indeed, realizing the danger of subjective elements in seeing, I have taken pains to have disinterested parties criticise my drawings from an inspection of the preparations.

Stage III.

The germinal vesicle retains its spherical form, and increases in size with the growth of the egg. Its size, however, does not seem to be con-

stant in eggs of the same size. It also retains its affinity for nuclear stains. The number of nucleoli remains about the same, and they retain their position at the periphery of the germinal vesicle. They still vary in size, and do not seem to grow perceptibly after their formation, being scarcely larger in the large egg, represented in Plate VII, Fig. 87, than in eggs like those represented in Plates IV, V, VI.

The nuclear reticulum remains visible as far as I have been able to trace the germinal vesicle in later stages. After the stage represented in Plate VII, Fig. 87, the egg becomes so filled with yolk that it is difficult to section it successfully.

The distance of the germinal vesicle from the cytocenter increases with the growth of the egg, while its distance from the periphery remains about the same, as is evident from an inspection of the plates. Compare, for instance, Figs. 86 and 87 with Figs. 70, 71, 75. From the very beginning, the germinal vesicle lies in the peripheral zone, between the subcuticular layer and the cytocœl, and continues to occupy that position even as late as those eggs represented in Plate VII, Figs. 84, 85, 86, 87. In Plate II, Figs. 34, 35, 36, 38, 39 and 49, 50, 51, the outer limit of the cytocenter, the cytocœl, is distinctly seen. Note that its relation to the germinal vesicle is about the same in all these cases. It intersects the germinal vesicle at its lower one-fourth. Comparing these figures with the very young eggs of the first stage, as, for instance, Plate I, Figs. 12, 14, 16, 17, it will be seen how closely these relations are maintained throughout the first and second stages. Comparing again these with the eggs of considerable size of the third stage, represented in Plate VII, Figs. 84, 85, 86, it will be seen that the germinal vesicle occupies the same relative position with reference to the cytocœl. The one striking difference between them is the increased distance between the cytocenter and the germinal vesicle. This is especially evident in Plate VII, Fig. 87.

The cytocenter is still visible in eggs as large as that represented in Plate VII, Fig. 87, and in much larger eggs (Fig. 88) where the cytoplasm is crowded with the regular yolk-bodies. The form of the cytocenter in these large eggs is variable. It is still very distinctly differentiated by orange G. (Plate VII, Fig. 86); by acid fuchsin (Fig. 87); and by hæmatoxylin (Plate VII, Fig. 88). In eggs like those of Plate VII, Figs. 84, 85, the cytocenter still retains much of the typical characters of the attraction sphere of younger eggs, it being as yet not invaded by the yolk-bodies. But in eggs like those of Plate VII, Fig. 86, the great increase of the yolk, both around and within it, nearly obscures it. The circular form is still maintained, and distinctly differentiated from all else it is doubtless a remnant of the denser central portion seen in

Plate VII, Fig. 85. So far as my observations extend on these larger eggs, the cytocenter exists wherever the germinal vesicle exists.

The yolk-nucleus is prominent in these eggs. It is especially conspicuous in eggs at the transition between the second and the third stage of growth (Plate IV, Figs. 68, 71; Plate V, Figs. 72, 73, 74; Plate VI, Fig. 78, 79, 80, 81, 82). It is, however, not confined to this transition period, but it is found in eggs of all stages of the second period of growth (Plate I, Figs. 23, 26; Plate II, Figs. 30, 33, 36, 37, 38, 39, 42, 44, 51; Plate III, Figs. 53, 54, 57, 58, 59, 60, 61, 63, 64). The principal characteristics of the second period of growth, besides the appearance of the nucleoli in the germinal vesicle, has previously been stated to be the granular condition of the cytoplasm; that of the third stage, the origin of the true yolk-bodies.

The yolk-nucleus has no such constant morphological feature as the germinal vesicle and centrosome or cytocenter. There is no apparent limit to the number that may exist in an egg (Plate III, Fig. 59; Plate IV, Fig. 69). The size varies greatly even in different sections of the same egg (Plate VI, Fig. 80). They are often circular in section and regular in outline (Plate III, Fig. 54; Plate II, Fig. 37), or they may be oval (Plate IV, Fig. 66); or they may be greatly elongated (Plate IV, Fig. 68); or they may be twisted (Plate II, Fig. 31; Plate IV, Fig. 70); or they may be very irregular (Plate IV, Fig. 71; Plate VI, Figs. 80, 83). In the smaller eggs, they are often located near the periphery (Plate II, Figs. 33, 36, 42; Plate III, Fig. 63; Plate IV, Fig. 68). I assume that these are the bodies that were seen by Clark, **20.** They are also found in the neighborhood of the cytocenter (Plate I, Fig. 23; Plate II, Figs. 37, 38, 51; Plate III, Fig. 54). But their greatest development seems to occur in the neighborhood of the germinal vesicle (Plate III, Figs. 57, 58, 59; Plate IV, Figs. 69, 70), and may partly surround the germinal vesicle (Plate VI, Fig. 79). It is often so close to the germinal vesicle as to make the hypothesis of continuity with the granular nucleoplasm extremely suggestive (Plate II, Figs. 37, 47, and Plate IV, Fig. 69, 70, and Plate VI, Fig. 79, 83). I can discover no law regarding its distribution throughout the egg, except that it usually occurs in that region of the cytoplasm which I have designated the cytocœl (Plate II, Fig. 31, 38, 51; Plate III, Fig. 57, 58, 59, 60, 61; Plate IV, Figs. 67, 69, 71; Plate V, Figs. 72, 75, 77; Plate VI, Figs. 78, 80, 81, 82, 83).

There are good reasons for believing that this yolk-nucleus is more or less fluid, and that it spreads throughout the cytoplasm sometimes by ordinary diffusion; but, at other times, by actual currents. These cur-

rents or whatever else it may be, sometimes leave a track or channel behind, in which the granules of the matrix are scarce or almost absent. Consequently the cytoreticulum is especially distinct. I do not know how to designate this effect except by the rather awkward term plasma channel.

These channels are rarely straight; they turn and twist in every direction. Consequently a longitudinal section of such a channel is rare. In Plate II, Fig. 31, is represented a plasma channel in the form of a long, bent and twisted body with an enlargement at each end. Another is represented in Plate IV, Fig. 68. If the granular substance of which this is composed should all flow toward one end, it would leave a temporary track in which the cytoreticulum would be evident. I take it that such a transfer of granular matrix actually takes place. Cases can be found where both longitudinal but more frequently transverse sections of such channels occur. Such an one is very evident in Plate VI, Fig. 78. The material having thus flown together would form a more or less spherical body, as appears in Plate IV, Fig. 66; Plate VI, Fig. 80, and Plate III, Fig. 54; Plate I, Fig. 23; Plate II, Fig. 37. It is evident from these figures, also, that several such spherical masses often exist in the neighborhood of the cytocenter (Plate I, Fig. 23; Plate II, Figs. 30, 37; Plate III, Fig. 54, etc.)

Plasma Channel.—Most interesting facts to me have been such appearances as those represented in Plate V, Figs. 72, 73, 74, serial sections of the same egg, where the plasma channel is actually continuous with the germinal vesicle. These figures are not at all exaggerated, incredible as it may seem. The channel is round in section. The very distinct cytoreticulum within this channel is certainly, so far as can be seen, directly continuous with the contents of the germinal vesicle. At the bottom of this channel the granular mass has accumulated, apparently while flowing out from the germinal vesicle and afterward divided into several currents. In Plate VI, Fig. 81, is another, somewhat elongated form, drawn from reconstruction of serial sections. In the different sections the granular mass forms a ring around the oval open space as is indicated in the drawing. This has been seen in other sections also. Most of the material here, it will be noticed, has become scattered in small, irregular bodies throughout the cytocœl, several such bodies also appearing close to the germinal vesicle.

A comparison of Plate V, Figs. 72, 73, 74, and Plate VI, Fig. 81, with Plate VI, Fig. 83, suggests that the latter is similar to the former, in that it is more or less spherical, and is, to all appearances, connected with the germinal vesicle. In this case, however, the granular substance

has not yet flowed out of it. The light areas might suggest, perhaps, that it is not a mere reservoir or a single channel into which the liquid substance is poured, which is also suggested by Plate IV, Figs. 69, 70. Its connection, real or apparent, with the germinal vesicle would be strong evidence in favor of the theory of nuclear origin were it not for the marked difference in staining between it and the contents of the germinal vesicle.

I have reasons for believing that it is not fatty in nature. The usual method of imbedding and mounting emulsifies the oil globules which arise in the egg during the formation of the true yolk-bodies, and causes them to disappear entirely in the prepared material, whilst, as I shall show presently, they are very large and numerous in material not so treated.

The stains which bring this yolk-nucleus most prominently into view are acid fuchsin, saffranin and eosin. With these stains it is more conspicuous than any other part of the egg. It often resembles archoplasm very closely. Its granular characteristics are most marked when stained with acid fuchsin and saffranin.

I have no reason to believe that this yolk-nucleus is at all permanent or that it simply accumulates in the cytoplasm as the egg grows. It may, apparently, be present or absent in eggs of equal size. Thus, compare, for instance, the serial sections a, b, c, d, e, Plate III, Figs. 57-61, with Plate III, Fig. 62, an egg of about the same size. The cytocenter is present in both, but not the yolk-nucleus.

The yolk first makes its appearance as definite spherical yolk-bodies when the egg has attained the size represented in Plates IV, V, VI. It is certainly very suggestive that the yolk-nucleus is so very prominent just before the yolk-bodies begin to form (Plate V, Figs. 76, 77; Plate VI, Figs. 78, 79, 80). Yet the yolk-nucleus is by no means peculiar to this stage of growth, as it occurs just as frequently in the very smallest eggs of the second period of growth (Plate I, Fig. 23; Plate II, Figs. 30, 31, 33, etc.).

The yolk-bodies appear as small, bead-like bodies in little vacuoles, one in each, arising between the subcuticular layer and the peripheral zone of the cytoplasm (Plate V, Figs. 72-74, and Plate VI, Figs. 78, 79, 80). At first they are few, with long intervals between them (Plate V, Figs. 72-74). Later they increase, both in size and in number (Plate V, Figs. 76 and 77).

They next arise in the cytocœl, forming a ring around the cytocenter which has now increased greatly in size (Plate VII, Fig. 84). This zone of yolk-bodies gradually broadens, encroaching, on the one hand,

on the peripheral cytoplasmic zone and, on the other hand, on the cytocenter. The yolk-bodies then form rapidly inward toward the central portion of the cytocenter, developing even in the central portion of it (Plate VII, Fig. 86). The yolk-bodies first formed in the cytocœl are the largest; and those latest formed in the cytocenter are the smallest at this stage. The yolk-bodies first formed at the subcuticular layer, although the oldest, do not grow so rapidly. They seem to stain differently from the larger spheres nearer the center of the egg (Plate VII, Fig. 86). In a very much larger egg, the cytocenter can still be seen, having now the appearance of a mass of granules (Plate VII, Fig. 87). At some distance from this, there is a zone forming a ring around the center in which the yolk-spheres are still very small and showing the original reticular cytoplasm filled with small yolk-bodies. Notice the light ring surrounding the cytocenter in Plate VII, Fig. 85. The same feature is visible in a very much larger egg, when the yolk-bodies have become very large and nearly uniform (Plate VII, Fig. 88, *i. cy. c.*). It is now a narrow ring, encircling the cytocenter about half way between the latter and the periphery of the egg, and consisting of closely-packed yolk-bodies of minute size and having considerably less affinity for the stain. Both in this stage and in the preceding the yolk-bodies first formed at the periphery have become quite large; smaller spheres have developed outward, so as to encroach on the subcuticular zone, and likewise inward. Yet the yolk-ring first formed has not been merged into that of the second, but is separated from it by a zone of minute yolk-spheres similar to those of the inner ring.

Comparing the yolk of these eggs with the yolk of eggs merely killed with the same preserving fluid, and preserved in 70 per cent alcohol, I found that there are certain bodies in the latter yolk which are not to be seen in the mounted section (Plate VII, Fig. 89). These bodies vary in size, but some of them are large enough to be seen with the naked eye. They are yellow to the naked eye. Under the microscope they appear white or transparent almost like water. They become especially prominent when iodine is applied to the preparation. This solution stains all the true yolk-bodies a deep yellow, but has no effect on the spheres under consideration. The yellow yolk-bodies, especially the smaller ones, seem to cling to the much larger white spheres so as to form clusters with the white spheres in the center. Smaller spheres or vacuoles can sometimes be seen inside the larger ones. Instead of a larger white sphere, there may be a bunch of very little ones having the same optical properties.

The application of chloroform has a peculiar effect on such a preparation. As soon as the chloroform is applied, the white globules, wherever

the chloroform comes in contact with them, break up into innumerable tiny droplets, that go spinning in all directions, setting up strong currents in the whole mass. In this way these bodies all disappear. I take this to be somewhat similar to an emulsion, and the white globules to be of a fatty or oily nature. It is doubtless the chloroform in the ordinary process of imbedding, possibly also the heated paraffine which is responsible for the absence of these globules in the mounted specimens.

The true yolk-spheres differ from these globules in being homogeneous throughout (Plate VII, Fig. 100). Others, slightly smaller, are finely granular (Plate VII, Fig. 99), while still smaller ones are coarsely granular (Plate VII, Fig. 101). Comparing the yolk-bodies in the order of their size, as Figs. 98, 99, 100, 101, it appears that the inner granules grow smaller as the yolk-spheres grow larger, till the homogeneous state is attained in the larger spheres. This might be taken to mean that the same sphere changes in this respect as it grows, were it not for the fact that many of the smaller spheres are as homogeneous as the largest, a fact which may mean that there are specific differences between the various spheres throughout their entire history.

The egg-membrane consists of an outer homogeneous layer which when torn has a fibrous appearance. Within this outer layer there is another one having radial striations (Plate VII, Figs. 84, 85, 86, 87). Surrounding the egg-membrane are the follicle cells forming a compact single layer of approximately equal cells (Plate I, Figs. 1, 2).

As the egg grows, it pushes out more and more from the germinal ridge, and becomes surrounded by a second and a third epithelial layer (Plate I, Figs. 1, 2). The second of these seems to be the original stroma cells surrounding the oogonia within the ridge. It remains quite closely applied to the follicle cells, but seems not to be organically connected with the follicle. This epithelial tunic, as well as the third or outer tunic, is richly supplied with blood-vessels. The third or outer tunic is more loosely applied, forming a loose bag, as it were, around the egg. It arises evidently from the peritoneal part of the stroma of the germinal ridge. When the egg is discharged from the ovary, it enters this outer bag, which serves to convey it to the oviduct.

On the Organization of the Egg.

Throughout the entire history of this egg, both the nucleus and cytoplasm exist. At first the cytoplasm is very much reduced, being apparently little more than an attraction sphere with archoplasm extending

part way around the nucleus. There is, outside of this, a thin layer of cytoplasm.

The fibrous nature of this cytoplasm is evident, especially in the neighborhood of the centrosome, which is the focus of the astral system. The astral system is a continuation of the cytoreticulum whose fibers consist of microsomes apparently imbedded in a less stainable substance. The network is imbedded in a hyaline matrix, the cytolymph.

This cytoplasmic structure is evidently continuous with a somewhat similar structure in the nucleus. The bead-like stainable bodies embedded in the linin network and which becomes aggregated into chromosomes, are, so far as their relation to the nuclear reticulum is concerned, similar to the cytomicrosomes. They differ, however, in their staining capacity, as is well known in the case of other eggs also.

Like the cytoreticulum, the nuclear reticulum is evidently suspended or imbedded at first in a clear matrix or karyolymph. In both the cytoplasm and in the nucleus the matrix becomes turbid through the formation of tiny granules. The deposit of these granules takes place in the nucleus slightly earlier than in the cytoplasm, and seems to be accompanied by the formation of nucleoli, just as in the cytoplasm it is accompanied by the formation of yolk-nuclei, and considerably later by the formation of true yolk-spheres.

Evidence tending to show that these granules belong to the matrix both of the germinal vesicle and of the cytoplasm, is afforded, in the first place, by the fact that the nuclear reticulum can be seen even when the egg is filled with yolk, and even so late as when the germinal vesicle lies close under the egg-membrane (Plate VII, Fig. 87), and in the second place, by such appearances as are represented in Plate V, Figs. 72, 73, 74, and Plate VI, Fig. 81, where the granules have temporarily accumulated in one spot, and have left the meshes clear behind them. Here the fibrous cytoreticulum comes again prominently into view. If this is due to a flowing movement in the interfilar substance, it should afford evidence in favor of the reticular theory of protoplasm, as contrasted with the alveolar. The fibrous structure of the cytoplasm becomes again prominent, also, in connection with the cytocenter.

I conclude that this reticulum, both of the nucleus and of the cytoplasm, is the real organized substance of the egg, and that, on the other hand, the matrix with its contained granules possesses no organization, no permanent form, but is like any other chemical mixture of organic substances, the culture medium, so to speak, of the organized substance.

This theory of the permanence of the reticulum of the nucleus and cytoplasm, about which there seems to be some difference of opinion, is supported by the facts observed in this egg regarding the permanency of the attraction sphere and cytocenter and the resulting polarity of the egg.

The Reticulum.—There is certainly good reason to be skeptical regarding the permanency of this reticulum, and consequently of its real morphological value. Reagents are often held responsible for its artificial production. To test the possible effects of reagents in this regard, I have made permanent preparations, by the ordinary histological methods, of the striated muscle of an insect larva, in which the longitudinal fibers and the transverse striations in their minutest details are beautifully shown. On examining the living larva with the same magnifying power, I found that I could see every detail about as plainly in the living contracting muscle. These details in the living muscle were not altered in the least in the prepared material except that the fibers and their verrucosities were made more conspicuous.

The Centrosome.—The regular arrangement of the microsomes and radial fibers, immediately surrounding the centrosome in the resting state, points to a primitive and permanent architecture in the midst of this complex system of fibrils. In my work on the egg of Limulus, **61**, I came to the conclusion that the vitalline-body in that egg is a direct continuation of the centrosome of the dividing oogonia, just as I have been forced here to believe that the cytocenter in the later stages of the egg of the tortoise is a continuation of the centrosome of the dividing oogonia. Wilson, **84**, has intimated that these bodies, like ordinary yolk-nuclei, may be the result of metabolic activity of the nucleus, and that the entire cytoplasm may be derived from the germinal vesicle. The evidence of continuity of the cytocenter with the centrosome is more conclusive in the egg of the tortoise, and it is, furthermore, so radically different from the yolk-nucleus as previously described, that it seems rash to insist on any identity between the two. I can readily admit that so much of the facts as could be shown in the plates of my work on Limulus was not sufficient to establish such a vital point, and that even all that I could gather in four years of continuous study of that egg was not equal to the amount of labor expended. In a prolonged study of this kind, one naturally, I suppose, forms certain general conclusions which cannot be gained from a mere inspection of the plates. Yet, what is evidently needed is positive, not negative evidence of normal not abnormal or pathological conditions.

The evidences, so far produced by writers, of the disintegration and disappearance of the centrosome are all of a negative rather than a positive nature. Negative evidence of such a body as the tiny granule of the centrosome, or even of the surrounding microsomes and radial fibers in the midst of a granular cytoplasm, may well create doubt rather than conviction, to say the least. The few cases of multiple centrosomes, with which we have been made familiar, were either admittedly pathological, or else would seem to be temporary aggregations of the cytoreticulum having no connection whatever with a normal centrosome. About all that can be said concerning pathological centrosomes, if they be centrosomes at all, is that they are what they are admitted to be, namely, pathological. Such evidence must be of doubtful value in estimating normal structures. And when a few such abnormal structures are made the foundation of a whole system of beliefs, as sometimes seems to be the case, what assurances have we that the whole system is not as abnormal as the foundations on which it rests? In the granular cytoplasm, like that of the egg, it is a comparatively easy matter to find centrosomes almost anywhere, especially if one has multiple centrosomes in the eye to begin with. Thus, in mounted sections of eggs like that represented in Plate VII, Fig. 87, a very regular and pretty radial system of fibers surrounds those large yolk-bodies that do not lie too closely packed for it to be seen. But who would say that these yolk-bodies are centrosomes, or that such a system is homologous to a true aster or even comparable to the cytocenter as seen in these eggs?

The evidence of the continuity of the centrosome of the dividing oogonia with that of the growing oocyte, is more satisfactory, it seems to me, in this egg than in the egg of Limulus, and has tended to strengthen my belief in the correctness of the views expressed in my paper on that subject.

That there is some constant relation between the cytoplasm and the germinal vesicle, and that the latter is not merely a chemical mixture, is suggested, first, by its constant position in the cytoplasm, its constant relation to the cytocœl, and hence the cytocenter; second, by its constant form, the persistence of the chromatin network, as well as the peripheral arrangement of the nucleoli. I can find no evidence that these nucleoli are influenced by gravity. No matter in what plane the germinal vesicle is sectioned, the nucleoli are about equally distributed at its periphery.

Chemical Processes.—One is almost forced to believe that the nucleoplasm, which makes its appearance after caryokinesis of the oogonia

on the reconstruction of the nucleus is due to metabolic activity of the chromosomes. The granules, which accumulate in this hyaline karyolymph in the second stage of the egg, seem also to be the result of chemical action of some kind.

It is claimed by many observers that chromatin passes out from the germinal vesicle in some eggs and becomes changed in the cytoplasm either directly into yolk, or assuming temporarily the form of yolk-nuclei, is either finally absorbed by the cytoplasm or else later converted into yolk. No satisfactory proof of this elimination of chromatin has yet been given. In the present case, it would be natural, perhaps, to infer that the yolk-nucleus in this egg has such an origin. Its relation to the germinal vesicle is such as to suggest such an origin. But its staining reaction is such as to render that interpretation doubtful. All parts of the germinal vesicle stain deeply in nuclear stains. With possibly the exception of borax carmine, the yolk-nucleus resists these stains more than any other part of the egg. Saffranin, acid fuchsin, and eosin differentiate it, but stain also the granules of the germinal vesicle. Saffranin is especially favorable in this regard. With these stains, therefore, one is strongly impressed by the similarity of the granules of the yolk-nucleus with those of the germinal vesicle, and would very easily be convinced that the presence of the yolk-nucleus in the immediate neighborhood of the germinal vesicle means the origin of the former from the latter. The application of carmine or hæmatoxylin, however, changes matters entirely, for, while the nucleus is deeply affected, the yolk-nucleus is not in the least affected by these stains. Wilson seems to recognize this difficulty, but avoids it by assuming that a chemical change takes place in the chromatin on entering the cytoplasm. It must be evident that such a chemical change would involve a contribution of some sort by the cytoplasm through which the chemical change, if such there be, is brought about.

In my work on Limulus, 61, I differentiated the substance in the neighborhood of the germinal vesicle by means of Lyon's blue. I further noticed that, in certain phases of the germinal vesicles of that egg, a clear zone appeared around it, which I took to mean the extrusion of karyolymph. I believe that interpretation is the correct one, and that the granular yolk-nucleus, even in this egg, is due to chemical union of karyolymph with some substance in the cytoplasm. It is, so far as I can see, an amorphous chemical substance in the cytolymph, more or less fluid and capable of a flowing movement between the fibers of the reticulum The frequency with which it occurs in eggs of the second stage, as well as its frequency in the cytocœl, and its scattered

condition would certainly suggest that it has something to do with yolk-formation as is frequently asserted. It is often found in rather small patches scattered throughout the cytoplasm, especially in the peripheral zone. And this condition seems to be most frequent at about that period in the egg's history when the true yolk-bodies arise.

There, are, however, other facts which almost preclude the possibility of this substance being converted into yolk-bodies directly. In the first place, the yolk-bodies arise, first between the peripheral zone and the subcuticular layer where the yolk-nucleus is rarely to be seen. In the second place, the yolk-nucleus exists in the egg when the cytoplasm is merely granular. The yolk-nucleus makes its appearance as soon as the cytoplasm assumes its granular appearance and may be found up to the time of true yolk-formation.

I am, therefore, led to the following conclusion regarding the yolk-nucleus: *It is a kind of metaplasm (or archoplasm) arising in the neighborhood of the germinal vesicle through the combined influence of the nucleus and cytoplasm.* From the place of its formation, it diffuses or flows throughout the cytoplasm where it serves as a culture medium of the living substance of the egg; in other words, it serves as food. The true yolk-bodies are a secretion of the living substance of the cytoplasm.

The growth of the egg seems to be due largely to the growth of the cytocenter, originally the centrosome. As this expands, the germinal vesicle approaches more and more the periphery, and is consequently greatly removed from the cytocenter formerly so near to it. It still retains its relation to the cytocœl, and this is possible because the peripheral zone becomes greatly thinned out owing to the expansion of the cytocenter and the accumulation of yolk-bodies within the latter. Reference to the plates will make this clear. By comparing the different regions of the cytoplasm in the earlier stages with the large eggs represented in Plate VII, the region of greatest growth is easily seen to be the central portion corresponding to the original sphere. The region of greatest growth is also the region where the greatest amount of yolk accumulates; hence the vegetative pole.

Polarity of the Egg.—The polarity of this egg is marked from the beginning and is determined by the relative position of the cytocenter and the germinal vesicle. In the young oocyte, immediately after the telophase of caryokinesis of the oogonium, the centrosome remains, as already stated, at one pole of the nucleus, now the germinal vesicle. The uniaxial feature of the spindle in that division remains in the young oocyte, being determined, in this stage, as in later stages, by the

position of the nucleus and centrosome respectively. The pole at which the centrosome is located becomes the vegetative pole, due, as I have shown, to the fact that it especially is the center of cytoplasmic growth.

The egg axis has no fixed relation to other parts of the ovary. I found this to be true, also, of the egg of Limulus. Nor does the eccentricity of the germinal vesicle show any fixed relation to the source of food so far as this can be determined in this egg. The egg of Limulus being related to a germinal epithelium rather than to a follicle, was especially favorable for the determination of that question, the source of food being there easily determined. In that egg, also, the eccentricity of the germinal vesicle bore no constant relation to the source of food. I was led in the study of that egg to the conclusion, which I am forced to accept here, that the egg axis is determined from the beginning by the position of the germinal vesicle and centrosome, and that neither gravity nor the topographical relation of the egg to other tissues has any important influence in the matter.

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REFERENCE LETTERS.

p. z.—Peripheral zone.
c. c.—Cytocenter.
cy. c.—Cytocœl.
i. cy. c.—Inner cytocœl.
i. y. l.—Inner yolk-layer.
o. cy. c.—Outer cytocœl.
o. y. l.—Outer yolk-layer.
sc. l.—Subcuticular layer.
st. m.—Striated membrane.
h. m.—Homogeneous membrane.
fl.—Follicle.
g. v.—Germinal vesicle.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Longitudinal section of a germinal ridge of the ovary of the tortoise, showing the stroma cells, nuclei of oocytes and caryokinesis of oogonia; also varying stages of growing oocytes with the beginnings of nucleoli in the germinal vesicle; the centrosome and its transformation into the cytocenter of the large eggs; also the variation in direction of the egg axis. The largest egg is a section at right angles to the egg axis showing the cytocenter, and the yolk-nuclei in the cytocœl. In the next largest egg are also seen various larger forms of the yolk-nucleus. Here also is seen the relation of the egg to the two outer epithelial tunics, in the outer of which a blood-vessel is seen.

FIG. 2. Transverse section of germinal ridge near the proximal end, showing various stages of the egg with its follicle, and its tunics as in Figs. 1, with the centrosome and sphere variously developed, and yolk-nuclei scattered throughout the cytoplasm of one of the large eggs. This shows also the variation in the egg axis.

FIG. 3. Horizontal section of the germinal ridge showing oogonia of various sizes, their final division by caryokinesis, and the formation of follicle and oocytes.

FIG. 4. Large oogonium showing surrounding stroma cells, a centrosome with two rings of microsomes—the essential structure of an oocyte at the beginning of growth.

FIG. 5. Oogonium with surrounding stroma cells and centrosome previous to division.

FIG. 6. Spindle stage of the oogonium, first phase of the division period.

FIG. 7. Two-cell stage of the oogonium previous to the second division.

FIG. 8. Second division of the oogonium leading to the four-cell stage.

FIG. 9. Four-cell stage of the oogonium after the second division.

FIG. 10. A group of cells probably resulting from the third division of the oogonia.

FIG. 11. The first differentiation of the oocyte from follicle cells, showing archoplasm and centrosome in the cytoplasm of the oocyte, and a central body in the nucleus of the follicle.

FIG. 12. Growing oocyte, showing traces of the centrosome and the crescent-shaped archoplasmic body at one pole of the nucleus.

FIG. 13. Section of growing oocyte at right angles to the egg axis through the centrosome and sphere, showing its central position in the cytoplasm, a very distinct sphere with its distinct central body, centrosome, in the center of a lighter area.

FIG. 14. Oocyte with pronounced polarity, showing its oval shape, and by the position of the circle of microsomes with indistinct central granule, its relation to the germinal vesicle, a relation which is maintained throughout its succeeding history.

FIG. 15. Oocyte showing the archoplasm forming a ring partly enclosing the germinal vesicle, probably also the first beginning of a nucleolus in the germinal vesicle.

FIG. 16. Oocyte showing the relation of the centrosome and sphere to the

germinal vesicle, a clear area around the centrosome, and an accumulation of granules in the nucleus, probably the beginning of a nucleolus.

Fig. 17. Oocyte more highly magnified, showing the nuclear reticulum, the bead-like chromatin bodies of various sizes, and in the cytoplasm a centrosome with its two rings of microsomes and their relation to the germinal vesicle.

Fig. 18. A more advanced oocyte with the first undoubted nucleoli in the germinal vesicle, and the cytoplasm filled with granules that obscure the microsome rings of the sphere.

Fig. 19. A still more advanced egg with a conspicuous sphere.

Fig. 20. Growing egg with a more or less fibrous archoplasmic sphere.

Fig. 21. Egg with large, almost homogeneous cytocenter, probably due to the kind of stain (hematoxylin) used.

Fig. 22. Egg showing a typical sphere consisting of a central body, centrosome, a clear zone surrounded by a circle of microsomes, which again is surrounded by a zone of radial fibers extending to the cytocœl, or outer circle of microsomes.

Fig. 23. Growing egg with true peripheral nucleoli in the germinal vesicle, and in the cytoplasm a cytocenter with astral radiations and two oval yolk-nuclei in its immediate vicinity.

Fig. 24. Egg with a rather large, homogeneous centrosome, surrounded by a zone archoplasm.

Fig. 25. Egg with a very large, apparently homogeneous protoplasmic cytocenter with a clear ring around it, and bearing a definite relation to the germinal vesicle.

Fig. 26. Egg with a distinct centrosome, cytocenter with astral radiations surrounding it; also in the cytoplasm a yolk-nucleus.

PLATE II.

Fig. 27. Egg with a large homogeneous cytocenter having very much the appearance of archoplasm.

Fig. 28. Egg with an indistinct circle of microsomes and astral radiations packed into a bundle on one side giving the cytocenter an elongated appearance, and extending nearly to the periphery of the egg.

Fig. 29. Egg showing the open cytocœl, the outer limit of the cytocenter, and its relation to the germinal vesicle. The peripheral zone of cytoplasm extending from this cytocœl to the periphery, is here clearly seen.

Fig. 30. Section of an egg showing a cytocenter in form of an aster with a large yolk-nucleus on one side. The multiplication of nucleoli and their variation in size is here evident.

Fig. 31. A somewhat larger egg with an elongated body, probably a combination of the archoplasm of the cytocenter with yolk-nuclei.

Fig. 32. Section of egg showing a simple spherical cytocenter, possibly a yolk-nucleus.

Fig. 33. Section of egg at right angles to the egg axis, showing cytocenter with astral radiations, and peripheral yolk-nuclei.

Fig. 34. Section of an egg showing cytocenter with two circles besides the inner one, and the relation of the outer circle, cytocœl, to the germinal vesicle; a slight indication of astral rays.

Fig. 35. Egg showing the cytocenter with a clear open space part way around it, the cytocœl, with some indications of radial striations in the cytoplasm of the peripheral zone.

Fig. 36. Egg showing a cytocenter with a centrosome, and a yolk-nucleus in its immediate neighborhood, and several at the periphery.

Fig. 37. Section of an egg showing an aster-like cytocenter, with a larger yolk-nucleus between it and the germinal vesicle and two opposite, not very distinct in the plate.

Fig. 38. Section of an egg, showing the cytoreticulum very distinctly and a cytocenter composed of circles of microsomes, in the midst of which are several yolk-nuclei.

Fig. 39. Section showing cytocenter with central granules; its relation to the germinal vesicle; yolk-nuclei at the periphery, and one near the center.

Fig. 40. Section of an egg showing cytocenter in form of a sphere, with clear central globule, circle of microsomes, and astral radiations; also many concentric zones in the cytoplasm.

Fig. 41. Section of an egg, showing the chromatin network in the germinal vesicle, numerous nucleoli, and a homogeneous cytocenter having the appearance of archoplasm.

Fig. 42. Section of an egg, showing cytoreticulum, a cytocenter with radial striations apparently continuous with the cytoreticulum, and bounded by a circle of large microsomes, the cytocœl; in the peripheral zone a large yolk-nucleus.

Fig. 43. Section of an egg with a cytocenter in which the central granules are most marked; a clearer zone surrounding it in which the granules of the cytoplasm are not so marked.

Fig. 44. Section of an egg showing three large yolk-nuclei and some smaller ones. The cytocenter of this egg is in another section, not here represented.

Fig. 45. Section showing a large cytocenter apparently homogeneous and feebly stained. It resembles archoplasm.

Fig. 46. Section of an egg, showing cytocenter with an unstained central globule, surrounded by archoplasm, and this again surrounded by an outer irregular ring of archoplasm shading imperceptibly into the general cytoplasm; in the germinal vesicle, nucleoli and chromatin network in the midst of a granular caryolymph or ground-substance.

Fig. 47. Section of an egg, showing cytocenter consisting of a central, granular spherical body, surrounded by a ring of similar substance, a lighter ring separating them; a similar ring bounding the outer circle from which radial striations are evident on two sides, a small yolk-nucleus close to the germinal vesicle.

Fig. 48. Section of an egg showing a homogeneous cytocenter with slightly darker central portion; in the germinal vesicle a headed nuclear reticulum.

Fig. 49. A section showing a cytocenter with a somewhat indistinct central portion and an outer zone of reticulated fibrils.

Fig. 50. Section of an egg of the tortoise showing germinal vesicle with peripheral nucleoli, chromatin bodies and a typical cytocenter resembling an attraction sphere with a central centrosome, and surrounded by an indistinct zone bearing a constant relation to the germinal vesicle.

Fig. 51. Section of an egg showing a cytocenter similar to the preceding as regards the number of circles or zones, but in which the reticulum of the outer zone is more distinct; also yolk-nuclei in the cytocœl or outer limit of the cytocenter.

PLATE III.

Fig. 52. Section of an egg at right angle to egg axis, showing the cytocenter consisting of a dark central body surrounded by a light ring which again is surrounded by a system of radial fibers like an aster; a number of yolk-nuclei in the cytoplasm.

Fig. 53. Section of egg showing the zones of the cytoplasm, the subcuticular, peripheral, the cytocœl and finally the zones of the cytocenter; yolk-nuclei in the cytoplasm.

Fig. 54. Section of an egg showing germinal vesicle with peripheral nucleoli, some of which are vacuolated; and besides the bead-like nuclear reticulum; a fibrous cytocenter resembling an aster; one large, round yolk-nucleus, and two smaller ones.

Fig. 55. Section of egg at right angles to the egg axis showing central body, cytocenter and the cytoplasmic zones around it.

Fig. 56. Section showing cytocenter in the form of an aster and a central body, centrosome and surrounding granular zone; a germinal vesicle showing peripheral nucleoli and chromatin bodies arranged in rows.

Figs. 57, 58, 59, 60, 61. Serial sections of the same egg, at right angles to the egg axis, showing (a) yolk-nuclei near the germinal vesicle; still more of them in (b) where the section passes through the center of the germinal vesicle, yet more in (c) where many of them are grouped around the pole of the germinal vesicle next to the cytocenter; the aster-like cytocenter in (d) surrounded by numerous yolk-nuclei; the central sphere (e) with radial fibres on one side and numerous yolk-nuclei arranged in a circle around it.

Fig. 62. Section of an egg, showing germinal vesicle, peripheral nucleoli, nuclear network, and a cytocenter consisting of a network of deliate fibres.

Fig. 63. Section showing germinal vesicle, with peripheral nucleoli, and bead-like chromosomes imbedded in a somewhat granular karyolymph or nuclear matrix. A large spherical cytocenter with distinct astral rays evidently continuous on one side with the cytoreticulum, at the periphery a large yolk-nucleus.

Fig. 64. Section of an egg showing germinal vesicle; cytocenter with archoplasmic zone and astral rays in the cytoplasm and numerous yolk-nuclei in the peripheral zone of the cytoplasm.

Fig. 65. Section of egg showing germinal vesicle and cytocenter; section not parallel with egg axis; cytocenter and germinal vesicle in different sections hence the closeness of one to the other. The cytocenter appears diagrammatic, but a true representation of very many of these centers, the large microsomes being slightly exaggerated.

PLATE IV.

Fig. 66. Section of an egg showing a single large vitelline-body of homogeneous substance resembling archoplasm occupying a somewhat eccentric position in the cytoplasm and staining very similarly to the granular matrix of the germinal vesicle.

Fig. 67. Section showing germinal vesicle with distinct nuclear reticulum, composed of spherical chromosomes of various sizes, and also numerous peripheral nucleoli; a cytocenter with a clear center and two archoplasmic zones; in the cytoplasm, also, numerous small yolk-nuclei arranged principally in the cytocœl.

Fig. 68. Section showing germinal vesicle with chromatin network; cytocenter with astral rays; an elongated yolk-nucleus connected with the periphery and a smaller one at opposite pole similarly connected.

Fig. 69. Section showing germinal vesicle; a cytocenter with central body surrounded by a denser zone, and an outer reticular zone. In the cytocœl, are numerous small yolk-nuclei and near the germinal vesicle a large irregular body staining like the smaller ones and apparently continuous with the germinal vesicle.

Fig. 70. Section of egg showing a reticulated irregular cytocenter; a distinct germinal vesicle in the neighborhood of which there is a conspicuous irregular yolk-nucleus apparently continuous with the granular matrix of the germinal vesicle.

Fig. 71. Section showing germinal vesicle with distinct nuclear reticulum, and peripheral nucleoli; a reticulated cytocenter with central condensation; a large round yolk-nucleus and several very irregular ones forming a more or less continuous mass in the cytocœl; several smaller yolk-nuclei in the neighborhood of the germinal vesicle.

PLATE V.

Figs. 72-74. Three serial sections of an egg, showing sections of the germinal vesicle, plasma channel, yolk-nuclei and cytocenter; a few true yolk spheres near the periphery close to the subcuticular layer.

Fig. 72. Section showing connection of the plasma channel with the germinal vesicle; the cytoreticulum in the plasma channel very distinct, and an irregular yolk-nucleus apparently connected with it; two other large yolk-nuclei and many small ones in the cytocœl, and true yolk-bodies at periphery.

Fig. 73. Section of same egg as 72, showing further the connection of the plasma channel with the germinal vesicle on the one hand and the yolk-nucleus on the other hand; several yolk-nuclei in the cytoplasm; a distinct cytocenter with evident astral radiations, showing the contrast between a true cytocenter and the yolk-nucleus; the first yolk spheres as in the preceding section.

Fig. 74. A section of the same egg as Fig. 72 and 73, showing yolk-channel; and the various forms of yolk-nuclei and their distribution.

Fig. 75. Section of an egg showing germinal vesicle; yolk-nuclei and their relation to the germinal vesicle and the cytocœl; a large oval yolk-nucleus; a reticulated cytocenter.

Fig. 76. Section of egg showing numerous small yolk-nuclei and their apparent connection with the germinal vesicle; numerous true yolk-spheres near the subcuticular zone.

Fig. 77. Section of egg showing nucleoli distributed apparently throughout the germinal vesicle, but really due to the fact that the section has passed near one pole of the germinal vesicle which on that account is smaller than

usual, numerous yolk-nuclei distributed throughout the cytoplasm; an irregular cytocenter; first yolk-bodies at the subcuticular zone of cytoplasm.

PLATE VI.

Fig. 78. Section of an egg showing germinal vesicle; numerous yolk-nuclei in cytocœl; a plasma channel; a reticular cytocenter; the first true yolk-bodies.

Fig. 79. Section of an egg through the germinal vesicle, nearly at right angles to the egg axis, showing the germinal vesicle and the relation to it of the large yolk-nucleus forming an incomplete ring around the germinal vesicle; numerous smaller yolk-nuclei scattered throughout the cytoplasm; also the first yolk-bodies at the subcuticular layer of cytoplasm.

Fig. 80. Section of an egg, showing germinal vesicle, peripheral nucleoli, and many small yolk-nuclei surrounding it; a conspicuous aster-like cytocenter, and numerous large yolk-nuclei both regular in outline and also very irregular and staining very deeply; yolk-bodies at the periphery.

Fig. 81. Section of an egg, showing a germinal vesicle with peripheral nucleoli and many small yolk-nuclei surrounding it; a plasma channel apparently connected with the germinal vesicle, as seen from reconstructed serial sections; numerous yolk-nuclei occupy the cytocœl and seem to surround the plasma channel as if issuing from it; a somewhat reticulated cytocenter having no similarity to the yolk-nuclei.

Fig. 82. Section showing the germinal vesicle with bead-like chromosomes, peripheral nucleoli; yolk-nuclei, and their relation to the germinal vesicle and to the cytocœl; a spherical, definitely bounded cytocenter with granular center and reticulated outer portion.

Fig. 83. Section showing germinal vesicle with nucleoli; and a large spherical yolk-nucleus connected with the germinal vesicle; numerous smaller ones in the cytocœl; a spherical cytocenter.

PLATE VII.

Fig. 84. Section of an egg, showing germinal vesicle; a fibrous cytocenter, surrounded by a zone of cytoreticulum; the order of yolk formation in zones—the outer yolk zone just under the subcuticular zone, and the second in the cytocœl.

Fig. 85. Section of an egg considerably larger than the previous, showing the relative increase of the two yolk zones and the relation of these to the germinal vesicle and to the cytocenter, *c.c.* The cytocenter is surrounded by a zone of protoplasm, the inner cytocœl, *i. cy. c.;* this again surrounded by the inner yolk layer, *i. y. l.*, this surrounded by the outer cytocœl *o. cy. c.*, followed by the outer yolk layer, *o. y. l;* outside of this the subcuticular layer, *sc. l.* Surrounding the subcuticular layer is the striated membrane, *st. m.*, outside of which is the homogeneous membrane, *h. m.*, the two constituting the egg membrane or chorion. The follicle, *fl.*, forms a single layer of cell surrounding the egg.

Fig. 86. Section of an egg more advanced than the preceding as is evident from the greater development of the yolk. The yolk-bodies have encroached on the cytocenter which is reduced to a crescentic mass of granular substance staining differently from the rest of the cytoplasm. The section shows

the relation of the yolk to the cytocœl and the relation of the latter to the germinal vesicle.

Fig. 87. Section of large egg, showing the germinal vesicle at the periphery, and the cytocenter, now an irregular mass of deeply staining granules. The outer zone has become narrowed by the encroachment of the inner yolk layer; the inner cytocœl still visible as a spongy zone of protoplasm.

Fig. 88. Section of a large egg, showing the cytocenter surrounded by fully developed yolk granules; the inner cytocœl, *i. cy. c.*, now a narrow zone of uniformily small yolk-bodies; the peripheral zone, *p. z.*, now filled with well developed yolk-bodies especially in the inner yolk layer, *i. y. l.*; the outer cytocœl, *o. cy. c.*, evident from the less perfectly developed yolk-bodies resembling those of the inner cytocœl, *i. cy. c.*; the outer yolk layer, *o. y. l.*, with well developed yolk-bodies: and the subcuticular layer, *sc. l.*

Fig. 89. Portion of the yolk of the largest eggs showing yolk formation and oil globule. Killed in the usual way, but not imbedded. Mounted on the slide and treated with iodine, showing oil globules unstained by iodine; arrangement of yolk-bodies around these large unstained globules, and their occasional breaking up into clusters of smaller globules often containing a large one in the center.

Fig. 90. Section of a cytocenter highly magnified, showing a small dark central body, surrounded by indistinct zones of granules, the outer less dense, and this again surrounded by a third outer zone apparently loosely reticulated in the meshes of which there seems to be a system of astral rays proceeding from the central mass.

Fig. 91. Section of a germinal vesicle in the early stage of development before true nucleoli have developed, and before the nuclear matrix has become opaque by the formation of granules.

Fig. 92. The ovary of the tortoise as seen with the naked eye, showing the various stages of the developing eggs. The smallest eggs mark the position of the germinal ridges lying between the larger eggs.

Figs. 93, 94, 95. Oogonia showing increase in size previous to division to form oocytes; stroma cells, with no follicle yet formed; nucleus in various stages of growth; the attraction sphere; the relation of the latter to the nucleus and to the outer protoplasmic zone of cytoplasm, the peripheral zone.

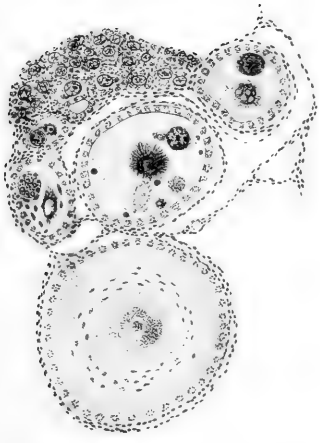
Figs. 96 and 97. Young growing oocytes, showing in the young germinal vesicle, bead-like chromatin network apparently imbedded in band of unstained linin substance; nucleoli forming in the interior, some of them already vacuolated; typical spheres with indistinct centrosomes, but distinct circles of microsomes with evidence of radial striations proceeding outward from the center; the relation of the sphere (cytocenter) to the germinal vesicle; the evident cytocœl, *cy. c.*, Fig. 97, separating the cytocenter, *c. c.*, from the peripheral zone of cytoplasm, *p. z.*

Fig. 98. Yolk-bodies of various sizes stained in iodine and showing in the larger a central body that is not so strongly affected by the stain and appearing like vacuoles but evidently some differentiated solid substance.

Fig. 99. Yolk-body composed of minute spherical granules throughout.

Fig. 100. Yolk-sphere homogeneous throughout.

Fig. 101. Smaller yolk-body filled with smaller yolk spheres but larger than those of Fig. 99.



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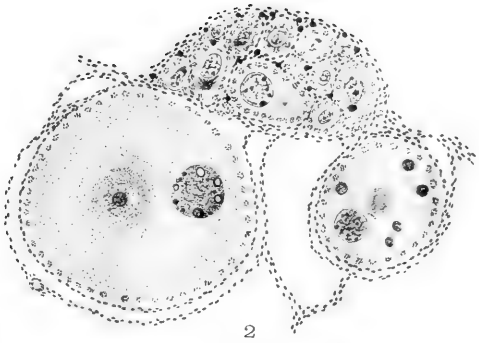

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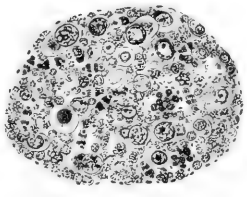

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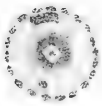

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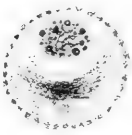

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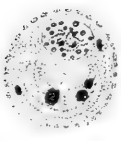

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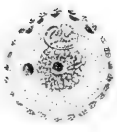

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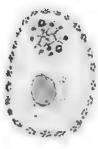

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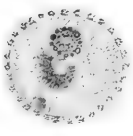

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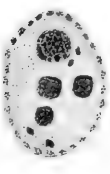

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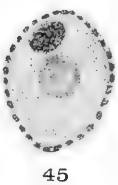

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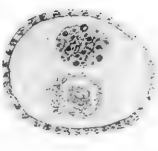

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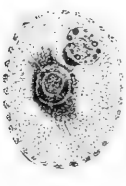

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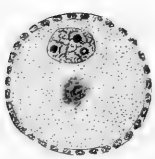

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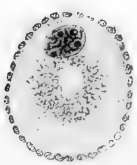

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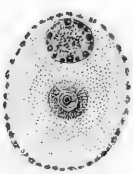

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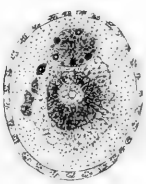

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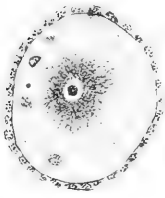

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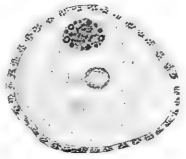

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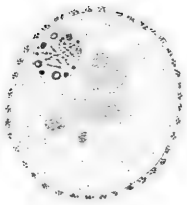

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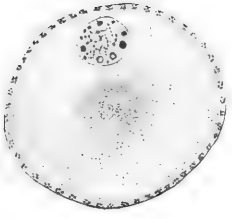

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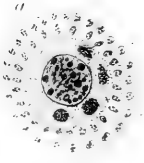

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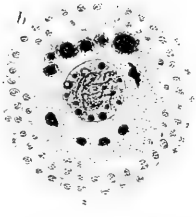

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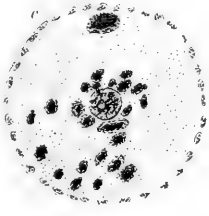

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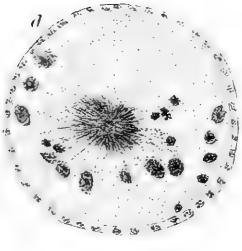

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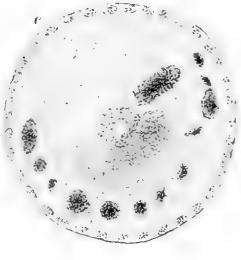

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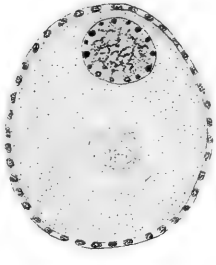

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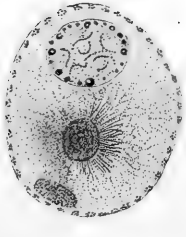

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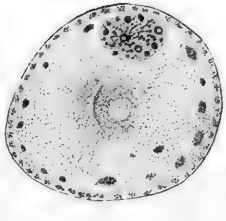

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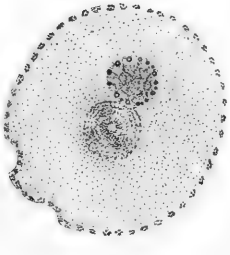

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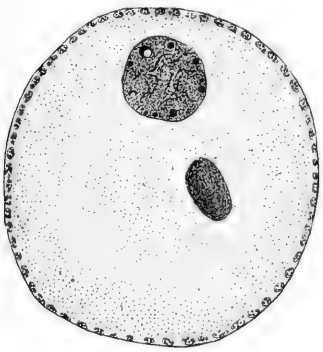

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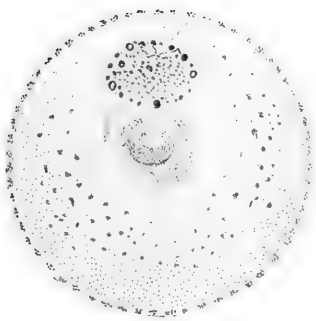

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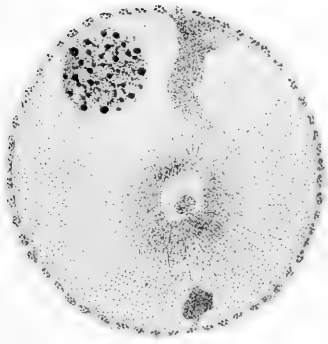

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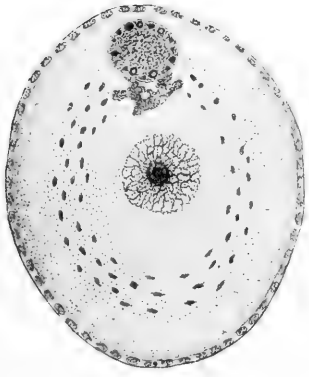

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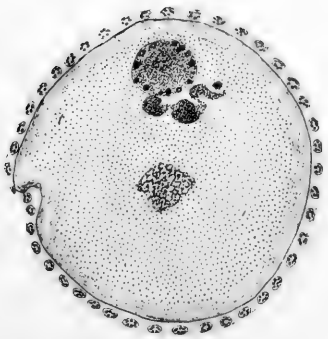

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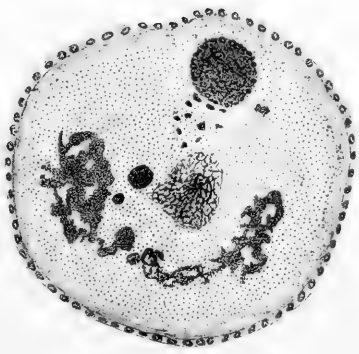

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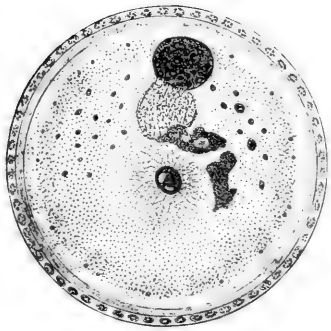

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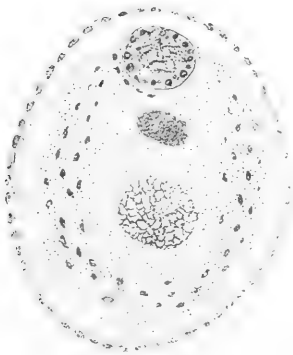

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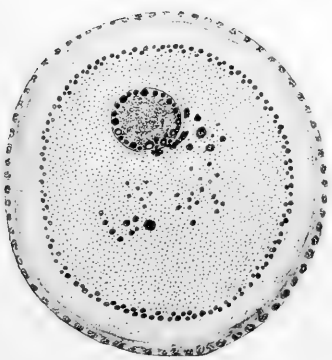

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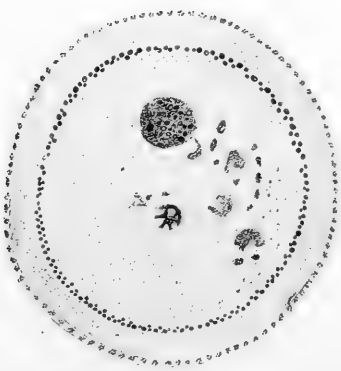

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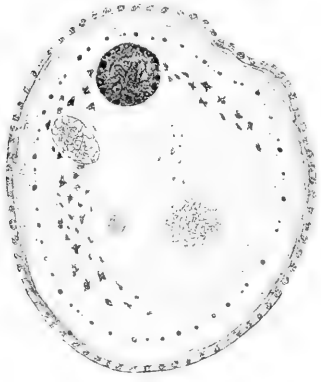

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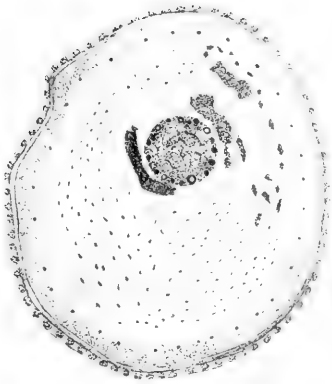

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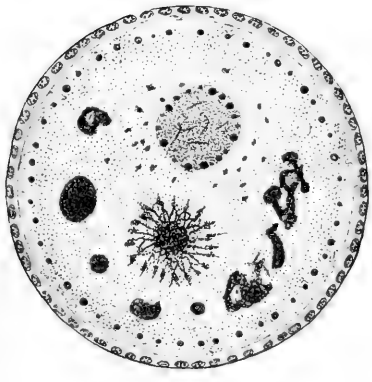

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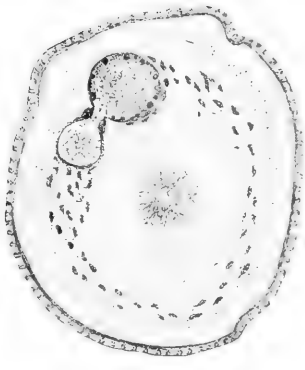

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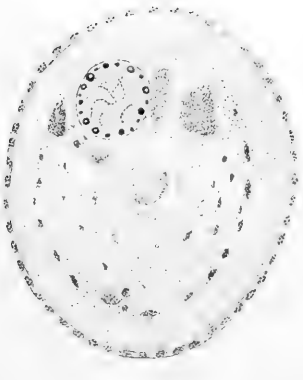

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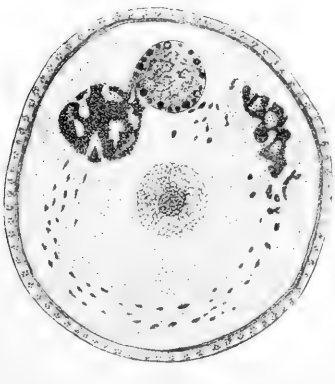

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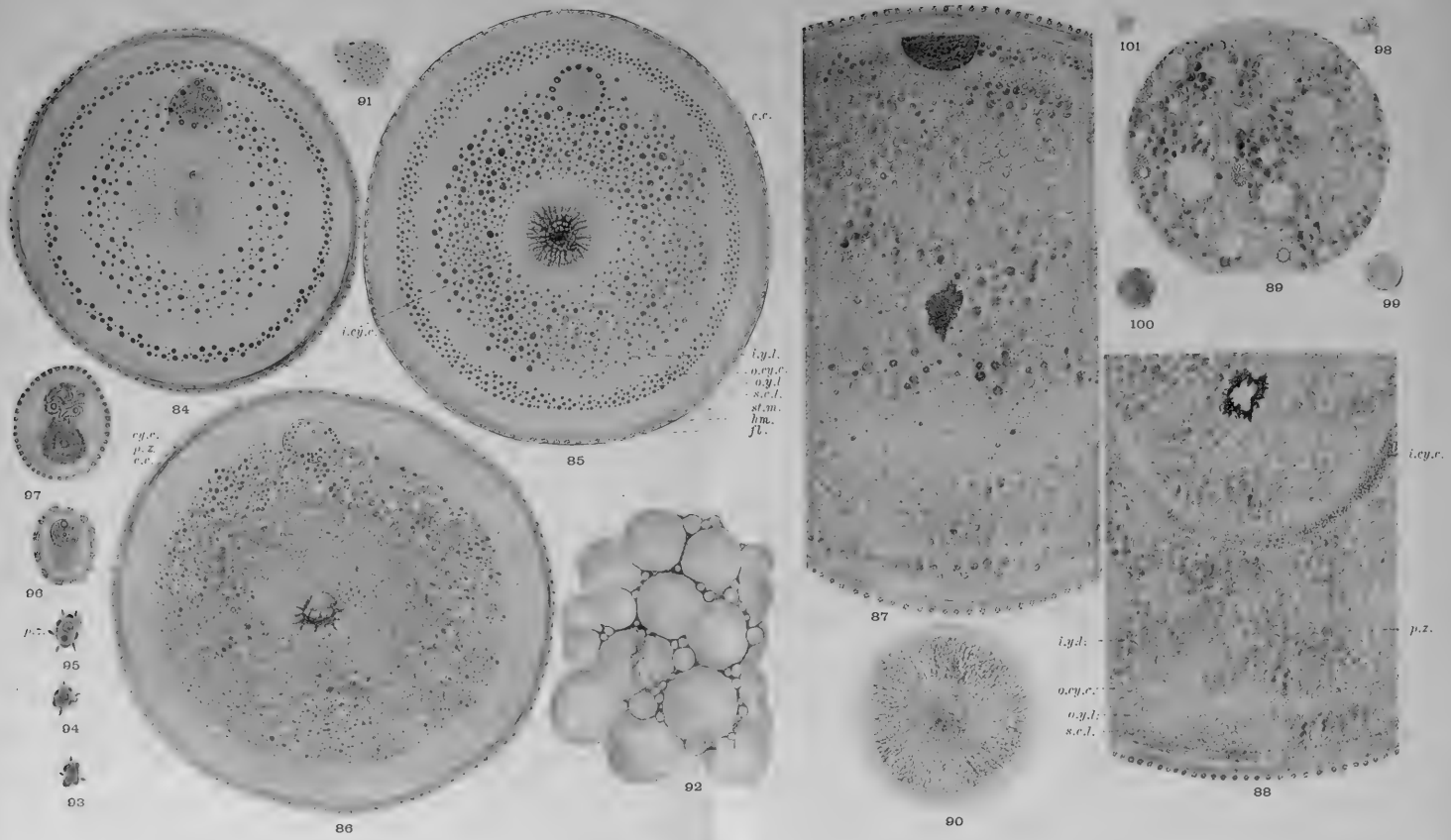

HELIOTYPE CO., BOSTON.

THE FERTILIZATION AND EARLY DEVELOPMENT OF THE PIGEON'S EGG.

BY

EUGENE HOWARD HARPER, PH. D.

WITH 4 DOUBLE PLATES AND 6 DIAGRAMS IN THE TEXT.

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The investigation, the results of which are here described, was proposed to me by Dr. C. O. Whitman, and the work has been carried on under his direction, being designed as part of a more general work upon the Natural History of Pigeons. My thanks are due to Prof. Whitman for his encouragement and suggestions and his assistance in obtaining the material.

In this paper the aim has been to get a view of that period of development of the bird's egg which has hitherto been scarcely touched upon, including the maturation, fertilization and early cleavage. Material was obtained from only one species, the common pigeon, Columba livia domestica. On account of the prolonged breeding season of pigeons and the ease with which they may be kept in confinement, they are

certainly better adapted to furnish material for studies of this sort than any other bird.

About the early development of the large meroblastic eggs comparatively little is known. This has remained true in spite of the thoroughness with which the embryonic stages of selachian and chick have been studied. As a result of the work of a number of investigators, chiefly Rückert, there is now a fairly complete general survey of the fertilization and early stages of the selachian egg. Observations upon the early development of the bird's egg are very few. Some of the early cleavage stages of the chick were figured by Coste, and Balfour contributed some observations. The internal phenomena of the egg during maturation, fertilization and early cleavage have remained an open field for investigation.

Upon the ovarian history of the bird's egg observations have been quite numerous. The paper of Holl, 90, upon the hen's egg may be mentioned as one of the most important.

The development of the large meroblastic eggs obviously presents numerous problems. In this paper the stages of the egg obtained are scattered over a considerable period, and present glimpses of various phases of maturation, fertilization and early cleavage. A few stages of the ovarian egg have also been introduced.

Methods.

The method followed has been to fix the whole egg before attempting to remove the germinal area. The oviduct is removed, the position of the egg being carefully noted, as this enables one to judge the approximate stage in development and determines the subsequent treatment in staining. The portion of the oviduct containing the egg is then cut off, immersed in the fixing fluid and slit open underneath the liquid. In case of an egg which is free in the body cavity, with some caution the body may be inverted over the fixing fluid, allowing the egg to drop out. The large ovarian egg may be fixed long enough to allow the fluid to penetrate the disc, then hardened in alcohol and the germinal area subsequently dissected out.

The choice of fixing fluids is somewhat limited, since many of them leave the disc too brittle to stand the subsequent treatment, and washing in water is undesirable. The picro-acetic mixtures have been chiefly used. Long fixation is not necessary or desirable, owing to the swelling of the yolk, which is apt to distort the disc.

It is well to cut out a considerable portion of the surrounding yolk with the disc and then to float this piece into a shallow watch-glass and

allow it to remain with the convex surface down in the watch-glass through the washing and hardening treatment. Lying on a flat surface tends to warp and often crack the disc. It should be trimmed evenly all around to overcome the tendency to curl in one direction. Occasionally the egg membrane will come off easily before or even after fixation. If not, the sharpness of the knife must be depended on to overcome this difficulty. Of course the knife should strike the inner side of the disc in its descent.

The abundance of the yolk and its obscuration of other structures would seem to make it desirable to use a stain which should mask the yolk as much as possible. In all but the fertilization stages the nuclei are surrounded by areas tolerably free from granules, and this is especially true of the sperm nuclei in their later divisons, which are surrounded by very large granule-free areas. For this reason the iron-alum hæmatoxylin stain is workable, and possesses besides an advantage in differentiating certain areas in the cytoplasm during its amœboid changes, which are less conspicuous with a stain which masks the yolk. The different degrees of extraction of the stain in the different areas of the cytoplasm is a highly desirable feature.

Some Observations on the Breeding Habits of the Common Pigeon.

The fact that the pigeon breeds so readily in confinement makes possible a close observation of its breeding habits. As is well known, the special instincts displayed in connection with reproduction are more highly developed in the pigeon than in the common fowl. These complex instincts are associated with monogamy, which reaches a type of development in the pigeon which is very high among birds. For example, the feeding of the young with "pigeon milk" may be mentioned. It is only with the earlier manifestations of the reproductive instincts prior to egg-laying that we are here concerned.

It might be supposed that in the case of a domesticated bird breeding readily in confinement, such as the pigeon, some approach might be made toward an exact method for determining the time of fertilization of the egg. The time of egg-laying is approximately definite, as all breeders know. The common pigeon ordinarily lays two eggs at a sitting, occasionally only one. The first egg is regularly laid late in the afternoon. The second egg will be laid early in the afternoon of the second day following.

It is evident that the determination of the time of fertilization of the second egg of the pair and the length of time taken in its passage

through the oviduct would be a simpler matter than to determine from external signs when the first egg is fertilized. It has been found that after the first egg is laid, in the course of a very few hours the second egg becomes detached from the ovary, is fertilized, and passes into the oviduct.

As stated above, the first egg is laid late in the afternoon. Early in the evening the second egg becomes free from its capsule in the ovary and enters the oviduct. In all cases observed this has taken place between seven and nine o'clock. The time taken in passing down the oviduct is relatively short, the far larger part of the time which elapses before the egg is laid being spent in the lower portion, known as the uterus, or shell-gland. It is evident that the second egg of a pair may be obtained at approximately any stage desired, beginning with a period a few hours before its fertilization.

The question arises whether there may be any criteria found for judging the time of fertilization of the first egg. It might be thought from analogy with the mammalia that the time of copulation would furnish such a criterion. It is quite plain from the regularity of the history of the second egg, as given above, that the exact period when the egg is freed from its capsule is dependent upon the female organization, and would be likely to occur at some definite period, probably at night. A moment's thought would, however, make it plain that it is highly improbable that a periodical receptivity, or period of heat, should be displayed by the female at this time. Experience of the writer has shown that any violent movement of the animal at this time is likely to result in a broken egg. Of course, such an egg as the bird's cannot be retained in the oviduct to await fertilization. Sperms are stored in advance, and the critical passage of the egg, after leaving its tough capsule in the ovary, through the oviduct till it acquires its coating of albumen and a shell, occurs at night when there are no movements of the animal to endanger its safety. The period of receptivity of the female is prior to this series of events. Copulation is repeated so often that no definiteness could be attached to it as a criterion. The question then arises whether the period of receptivity of the female has any definite duration, so as to indicate in this way when the maturation of the egg is taking place. From analogy with the mammal and with many birds, such as the common fowl, we commonly think of ovulation as exclusively a female function, going on regardless of whether the eggs produced are fertilized or not. Thus the common fowl produces unfertilized eggs regularly in the absence of a male. In the pigeon, however, ovulation is delayed until mating. When a mature pair ready

for mating are put together, egg-laying ordinarily ensues at the end of a rather definite period, at the least eight days. The female functions are held in abeyance till the proper stimulus is received from a mate. The maturing of the egg is so exclusively a female function that it seems odd at first thought that an apparent exception should occur to the rule. Of course, we know that the final maturation of the egg, or the giving off of the polar bodies, awaits in most animals the act of fertilization. But here the effect is produced upon the egg by the entrance of sperms. How mating itself and the act of copulation could influence the ripening of the egg in the ovary is another problem. In this connection the curious fact must be mentioned that two female pigeons placed in confinement together may both take to laying eggs. The function of ovulation is in a state of tension, so to speak, that requires only a slight stimulus, "mental" apparently in this case, to set the mechanism to working. At any rate, it is impossible to regard the presence of sperm in the oviduct as an essential element of the stimulus to ovulation, although it may have an important influence in the normal case. Our attention is directed to the various and complex instincts of the male which come under the head of courtship, both before and after mating is effected, as furnishing a part of the stimulus to the female reproductive organs.

Phylogenetic considerations would lead us to consider the peculiar habits of the pigeon as recently acquired. The retention of ova in the unmated female, is in particular not very firmly fixed, as the facts stated show. The habits of the common fowl are certainly more primitive. In monogamous birds it might be expected that the function of ovulation would be adjusted so as to take place only after mating, inasmuch as it is probable that in a state of nature mating may be delayed for various causes, and the production of an unfertilized egg is no trifling loss, as in the mammal. In polygamous birds mating is sure to occur, and the female functions may be adjusted for continuous ovulation, with the practical certainty that in nature no unfertilized egg will be produced.

The complex reproductive instincts of the pigeon, displayed in their highest form in the male, are matters of common observation among those who have observed pigeons, and need not be dwelt upon at great length.

As is well known, the strutting of male pigeons is not simply a feature of courtship and rivalry among males. It is continued until egg-laying begins, and is accompanied by a less active similar manifestation by the female. It is in fact an accompaniment of the whole period

from mating to egg-laying, during which copulation is of frequent occurrence.

There is an act which regularly precedes copulation, in which there is an apparent regurgitation of some secretion by the male which is taken from his throat by the bill of the female, in somewhat the same manner as the young birds take their food. It is a less violent manifestation than the feeding of the young, however. It is easy to see that here may be one of the sources of indirect stimulation to the female reproductive organs.

The male has the habit of frequently taking to the nest and calling the female by emitting a low growling noise and gently vibrating his wings. It is evident from a consideration of the complexity of these and other instincts, such as nest-building, that the initiation of reproductive activity in the female can ordinarily only be dated from the time of mating, or from the resumption of activity by an already mated pair. The female pigeon is either a very dull bird or a very exacting one, requiring constant attention and flattery to rouse her to her proper functional activity, or else the male must be accused of greatly magnifying his office.

There is a possibility that the nesting habits of the female could be used as a clue to the time of egg-laying. The female has the habit of sitting on the nest occasionally for some time before the first egg is laid, but in practice this has not been found to give sufficiently definite data.

No certain method has been found for determining the time of fertilization of the first egg. By making use of the second egg, any stage after or shortly before fertilization may be obtained. This method has the disadvantage of yielding only one early stage of the egg from each bird. The first egg when laid has reached the close of the segmentation period. The second egg would remain in the oviduct nearly forty-eight hours after the first was laid. To obtain a series of the late ovarian eggs is more a matter of chance.

In elasmobranchs and reptiles a considerable number of eggs are found in the oviduct and all in nearly the same stage of development. The greater certainty with which the pigeon's egg may be obtained is a compensatory feature, when we are considering the relative difficulty of obtaining material for a study of these forms.

The Fertilization of the Egg and Its Passage through the Oviduct.

The passage of the egg through the oviduct until it acquires a thin shell within the lower portion, or shell-gland, is a nocturnal function.

That, as such, it is adapted to secure the safety of the egg is evident from the thinness of the egg membrane when it leaves its tough ovarian capsule and its consequent liability to be ruptured at this critical period. Careful handling is necessary to secure the egg at this time. The capsule of the egg splits along the pole opposite to its attachment in the ovary. A gradual thinning out of the capsular wall occurs along the line of splitting, causing a pale streak across the egg. The vessels of the capsular wall are at this time highly charged with blood. Two such pale streaks across the egg have been seen at right angles. During the rupturing of the capsule, the egg bulges out in various places, producing an irregular appearance with several protuberances. Inasmuch as the wall grows quite thin during the process, it is quite possible that the spermatozoa may be able to penetrate and reach the germinal vesicle before the egg leaves its capsule. When the egg escapes, it is found well surrounded by a thin albuminous liquid with which the body cavity at this time is charged. It is like the albuminous secretion of the oviduct, except that it is much thinner. This liquid serves both as a medium for the spermatozoa, as stated by Balfour, and as a support to the egg at this critical juncture, when it is invested by only a very thin membrane.

The egg membrane or yolk membrane is about 3.5μ in thickness. The outer margin of the cytoplasm is somewhat denser and also takes on something of the character of a membrane. In some preparations this is found actually separated for a little way from the underlying cytoplasm. But for the most part it appears like a very thin non-separable layer.

The egg membrane appears structureless. It seems to increase in tenacity, since, when the egg is first set free from its capsule, it is very easily ruptured. The flattening of the yolk from its own weight in the fixing fluid is enough to cause the rupturing of the membrane. The increase in tenacity later may be the effect of the deposition of closely adhering layers of albumen.

The egg is clasped by the funnel-like mouth of the oviduct, which at this time has been observed to display active peristaltic contractions, as if in the act of swallowing the egg. The contractions were confined to the funnel portion of the oviduct. The fact as stated rests upon a single observation. As the transition from the funnel to the glandular portion is abrupt, it would seem that the egg must be engulfed by muscular contraction, but after it is within the glandular portion of the oviduct it is driven simply by ciliary action along its spiral course through the oviduct, as has been stated by Cushny, 02, in regard to the hen's egg. The peristaltic motions were sufficiently active to be unmistakable. The

desire to obtain the egg interfered with the continued observation of the movements, and it is not known how long they might continue. Morgan, 97, states that the old view that the frog's egg is swallowed by peristaltic motions of the infundibulum of the oviduct is probably mistaken, and that the egg is doubtless driven along its entire course by ciliary action. The oviduct of the pigeon is usually from twelve to fifteen inches in length, but sometimes over twenty inches. The funnel portion or infundibulum is less than one-fourth of the entire length; the glandular portion which secretes the albumen is a little less than one-half of the ordinary length. The remaining portion, the uterus or shell-gland, is separated from the preceding part by a definite constriction.

The entrance of spermatozoa is previous to the time when the egg is clasped by the funnel of the oviduct. An egg at this stage contains numerous sperm nuclei which have undergone considerable transformation and others in various early stages of transformation. Hence the entrance of spermatozoa must take place as soon as the germinal disc is exposed by the rupture of the follicular wall. This may be while the egg is still attached to the ovary, but the point has not been definitely ascertained.

The stage of development reached by the egg at any time is indicated approximately by its position in the oviduct. Thus the polar bodies are given off within the proximal part of the glandular portion, and cleavage begins just about as the egg enters the shell-gland. The passages through the upper portion of the oviduct in which the albumen is secreted is relatively rapid. The following table gives some data for an estimate of the time.

Beginning of first maturation division	.....	7:40- 9:00	P. M.
" " second " "	.....	7:45-10:15	P. M.
" " first cleavage "	.....	10:30-12:30	P. M.
" " second " "	.....	12:30- 1:00	A. M.

From such data only a rough estimate can be made as to the time elapsing between the impregnation of the egg and the first cleavage. Balfour, 85, states that in the fowl cleavage of the egg begins just before it enters the shell-gland. There is consequently a close similarity between the pigeon and the fowl in this respect. Since the absolute length of the oviduct varies in different birds, it would hardly be expected that the same relative position in the oviduct would generally be reached by the egg at the same stage of development. As a matter of fact the observed cases so far have been so close to the average as to furnish no evidence as to variation in this respect.

Some Late Stages of the Ovarian Egg.

The growth of the ovarian egg may be roughly divided from one point of view into two periods. The first is a long one of very slow growth, the second is a short period in which the main increase in size of the egg is effected. Two eggs mature at a time, occasionally only one. The second pair in order of development are usually quite small, the larger being ordinarily several millimeters in diameter when the first pair are mature, but it may be half-grown occasionally at this time.

The full-sized egg in its capsule is nearly an inch in diameter. In Fig. 1 is shown the nucleus of an egg 1.4 mm. in diameter. It measures 238μ in diameter, and, being nearly spherical, is greater in volume than the nucleus of an egg which was in the midst of its rapid growth and 15 mm. in diameter. The latter nucleus is lens-shaped, flattened against the follicular envelope, and its diameter is 378μ (Fig. 3). In a smaller egg 12 mm. in diameter the nucleus is shown in horizontal section (Fig. 2b). Its greater diameter is 329μ. The ground substance of the nucleus or germinal vesicle is of a finely alveolar character, appearing under a low power to contain only a few scattering deutoplasmic granules. Under a higher power, however, it is seen to be thickly studded with microsomes of the same character as the larger granules. The chromosomes are in a group in a somewhat eccentric position, surrounded by a system of radiations. Apparently pairs of dumbbell-shaped dyads are lying side by side. They are unequal in size, three of the pairs being considerably larger. Two of the pairs are crossed, lying very close together (Fig. 17). There are numerous glistening refractive bodies scattered among the chromosomes, some small and some in vesicular masses. At the center of the germinal vesicle is a considerable amount of chromatic staining material in the form of short threads, and also a group of rounded bodies like nucleoli lying underneath them. The nucleoli in some cases have short remnants of chromatic threads clinging to them.

A later stage is shown in Figs. 4a and b. In Fig. 4a the whole germinal area of this egg is shown, the germinal vesicle enlarged in Fig. 4b. This egg was the older of a pair, the nucleus of the younger of which is shown in Fig. 3. A comparison of the size of the nuclei shows a great diminution. The wall of the nucleus is seen to be breaking down. Its contour is no longer regular, but it has shriveled up and retreated from its manifestly former position. The disintegrating wall is surrounded by a zone of the nuclear ground substance. The diameter to the outer limits of this zone is 210μ, showing an invasion of the yolk

into the area formerly occupied by the germinal vesicle. The retreat of the nuclear wall is unlike ordinary plasmolysis from fixing agents. There is no vacant space left from the shrinking of contents. Such plasmolysis is evident in the case of the younger egg of the pair, Fig. 3, indicating a more watery condition of the nucleus in the younger and still rapidly growing egg. In this egg there are no remnants of threads, except for slight indications upon the more or less rounded nucleoli. The refractive bodies previously mentioned are present. The chromosomes are shortened as compared with the former instance and are irregularly placed. The deutoplasmic microsomes in the nuclear ground substance are less prominent owing to a greater extraction of the stain.

Underneath the germinal vesicle is a core of lighter staining material, extending inward to the bed of white yolk. The whole germinal area under a low power appears very finely granular compared with the coarse underlying yolk.

The next stage obtained is that of an egg which in the ordinary course of events would have become free from its capsule and passed into the oviduct in the course of several hours. To be more definite in this instance, the egg was taken from the ovary at 7:00 P. M., the first egg having been laid in the afternoon.

The cross section (Fig. 5) shows the equatorial band of chromosomes lying obliquely to the surface of the egg at the margin of the deutoplasmic area. There is an accumulation of a liquid substance at this point between the follicular wall and the granules, which, as in other eggs, may be called the perivitelline liquid.

The area occupied by the germinal vesicle is obliterated. There is very little if any difference in the appearance of the germinal area at this point, except for the perivitelline liquid above mentioned, and a much greater accumulation of a substance having seemingly the same character directly underneath the finely granular layer. This body of more liquid protoplasm appears in structure and staining properties like the contents of the former germinal vesicle. It fills a wider area, however, and is bounded beneath by the coarsely granular yolk. Two eggs were obtained at this stage, both showing the same appearance. It does not appear like an artefact, and though peculiar to this stage of the egg, it seems to be definitely related with later changes in the fertilized egg.

No stages were obtained between those shown in Figs. 4b and 5. Fig. 5 shows the acme of development of the ovarian egg. If the accumulation of granule-free protoplasm underneath the granular layer of the disc is derived from the contents of the germinal vesicle, as its appearance would indicate, it seems as if a centripetal movement of this sub-

stance had taken place simultaneously with a lateral invasion of the granular protoplasm. Possibly the path of this centripetal movement is indicated directly beneath the contracting germinal vesicle in Fig. 4b. Fig. 5 represents the stage supposedly when activity in the egg has reached a minimum. When activity is resumed in the maturation stages attention will be called to the fact that the underlying bed of granule-free protoplasm has disappeared and in its place is a cone-shaped active area (Fig. 8) extending clear to the periphery of the egg with the spindle at its apex. This would seem to indicate a centrifugal movement of the granule-free area at the time of giving off of the polar bodies.

The spindle is fully formed in this egg, although its oblique position makes it difficult to recognize the achromatic structures. The equatorial band of chromosomes shown in Fig. 18 is from the other of the two eggs above mentioned, and the section was almost parallel with the equatorial plate. The chromosomes appear as tetrads of unequal size. There is an appearance peculiar to the first polar spindle to which attention is called. There are within the circle of chromosomes and lying in the same plane, a number of deeply staining granules at the nodes of the linin network. They plainly differ from the deutoplasmic granules outside, having the staining properties of chromosomes or centrosomes. There are four or five especially large ones at the center.

Nucleoli in the Ovarian Egg.—The nucleoli which have been described in this later part of the ovarian history are, as has been stated, evidently derived from a chromatic network which becomes aggregated into rounded masses, and these soon undergo dissolution in the form of refractive bodies. Lebrun, 02, describes nucleoles derived from the granular chromatin which are present at the first appearance of maturation in the egg of Diemyctilus and speaks of their propensity to fuse together.

In the amphibian egg, King, 01, mentions the occurrence of such refractive bodies in the germinal vesicles. They are described as "yellowish green refractive bodies," which result from the disintegration of nucleoli. It seems likely that the small refractive bodies among the chromosomes are remains of nucleoli which are nearly disintegrated. The larger aggregations of refractive bodies and nucleoli found elsewhere in the germinal vesicle are in an earlier stage of the disintegrating process (*b* and *c* in Fig. 4b).

The nucleoli which change to refractive bodies and disappear have a different fate from the nucleoli in the previous history of the germinal vesicle. According to Carnoy and Le Brun, in the amphibian egg the nucleoli are aggregations of the chromatin network, which at definite periods break down and give rise once more to a chromatic thread. They

are a resting stage of the chromatin. According to the opinion of Wilson, 00, such nucleoli are to be regarded as chromatin masses distinct in nature from true nucleoli or plasmosomes. In the smaller pigeon ova such net-knots of chromatin are frequently seen, looking like the beginning of the formation of a nucleolus or the contrary, the unrolling of one to form a thread (Figs. 1a and b). But better evidence on the nature of these chromatin nucleoli may be obtained from other material than the pigeon. Without going too far afield from the purpose of this paper, it may be mentioned that the ova of the sparrow in the winter condition give an excellent example of chromatin aggregated into the form of nucleoli. There is an almost entire disappearance of the chromatin network and a large and variable number of nucleoli having the staining reaction of chromatin. This phenomenon is accompanied by a watery condition of the nucleus as shown by the great plasmolyzation from fixing agents. The eggs may be fixed so as to show no distortion, but the nuclei are invariably plasmolyzed.

This condition disappears when the growing season recommences in March. The chromatin threads reappear and the nucleus is no longer so easily plasmolyzed. The difference between the pigeon and the sparrow ova is accounted for by the fact that the ovary of the sparrow is in a resting state in the winter, while the breeding season of the pigeon, in comfortable quarters, is continuous except for a slight cessation in the fall, during moulting. If the view of Carnoy and Le Brun is correct, the evidence for the continuity of the chromosomes through the ovarian development alleged by Born, 94, must be mistaken.

A frequent appearance found in the pigeon ova was that of pale, broken-down nucleoli, looking rather like the "shells" of nucleoli, either inside the nucleus or outside close to the membrane. This appearance is entirely different from that of the previously described refractive bodies. Such bodies are described by Carnoy and Le Brun in the amphibian egg.

The Fertilized Egg.

Maturation Divisions.—The earliest stage of the fertilized egg obtained is shown in horizontal section in Figs. 6a and b. The surface appearance of the disc of such an egg is shown in Fig. 6. The slightly oval disc has a greater diameter of 3.5 mm. It is divided into two zones quite clearly distinguished in opacity, the outer zone being due to the abrupt thinning out of the fine granular matter of the disc. With a hand-lens the region of the nucleus may be made out in the living egg as a spot surrounded by a lighter ring or halo, the "fovea." The whole affected area surrounding the nucleus is shown in Fig. 6a. The nucleus

is in the center of a granular area which is surrounded by a hyaloplasmic zone. The inner ring of the zone of hyaloplasm is quite free from granules and here the sperm nuclei are imbedded. Outside of the clear ring the cytoplasm is less densely granular and there is an appearance of watery rays or channels passing out. The diameter of the affected area is about .5 mm. One side is more vacuolated and hyaloplasmic than the other, which fact will be recalled in connection with later appearances during development. There is a rather clearly marked ring which is not like the hyaloplasmic ring in appearance, but is filled with a ground substance having a more finely alveolar structure. This ring, which may be called the "polar ring," will be better described in connection with vertical sections of the disc. The sperm nuclei shown in this section are in an advanced stage of transformation, and their identity with entering sperms must be discussed further later on.

The egg nucleus is in the equatorial plate stage (Fig. 19). There are eight apparent tetrads (pairs of dyads) in a ring, the diameter of which is greater than in the mature ovarian egg. The chromosomes also are larger, and are of unequal sizes as before. The central spindle granules are present, lying in the plane of the chromosomes, at the nodes of the linin network. The central group of larger ones is conspicuous, as in the previous instance. It may be again stated that these granules have not been found in any other spindles than the first polar, and, as already mentioned, they present a similar appearance in the four observed cases. They would seem to be concerned in some way with the formation of the first polar spindle, and may indeed be condensations of the linin network at the foci of the system of radiations surrounding the group of chromosomes before the formation of the spindle (see Fig. 17), though this may sound like a rash suggestion, since the stages in the formation of the spindle are yet to be observed. If they are "accessory" chromatin material, they evidently do not undergo dissolution like the chromatin nucleoli.

The nuclei embedded in the hyaline zone are all of similar structure and staining properties. They vary in size from four to seven μ. They are of irregular shapes and do not have any bounding membrane. No asters have been found, but want of material has prevented the use of various staining methods. Those which have penetrated deeper are somewhat larger. Stages in the transformation of the sperms are shown in Figs. 7a-h. The entrance of sperms seems to take place anywhere within the affected area, but those which enter the hyaline zone seem to undergo a more rapid development. The fate of the great majority of sperms can best be inferred in lack of direct evidence from the numbers found

in the fertilization stages, where in the cases obtained the number was from 12 to 25. Apparently those not entering at the right time and place meet with some unfavorable influence hindering their development.

In the perivitelline liquid are found numerous large cells from the follicular membrane of the ovarian egg (Fig. 7i). These cells are without walls, but the nucleus and accompanying body of cytoplasm bears an unmistakable resemblance to the follicular cells. Associated with them are found also blood corpuscles. From their size and appearance they may be distinguished from the sperm nuclei, since they are of a much greater order of magnitude. There is much nuclear debris which is evidently derived from these cells also present in the perivitelline space, showing that their fate is to degenerate. Rückert, 99, has found the same "inwandering follicular cells" in the selachian egg.

A vertical section of the germinal disc during the maturation stage is shown in Fig. 8. The egg was taken from near the beginning of the oviduct. The section contains the second polar spindle, and first polar body, situated at a slight depression in the surface of the disc. The vertical section of the germinal disc shows that the central or affected area has the shape of a cone with the spindle at the apex. The distinguishing characteristic of the affected area which mark it off from the surrounding homogeneous appearing disc, is its lighter staining property. This seems due both to the relative fewness of the granules and the greater extraction of the stain from those present. The protoplasmic ground-work is apparently more watery, and vacuoles are very numerous. The finely granular material of the disc surrounding the affected area retains the stain with great tenacity. The deeply-staining layer is quite sharply marked off from the underlying yolk, which loses its stain completely. The deeper yolk composed of very large granules, retains the stain, and thus there is a lighter area sandwiched between two dark staining regions. This may be seen in the section of the ovarian egg (Fig. 4a). The "polar ring" mentioned previously is seen to be shallow, appearing in cross-section as two lighter staining V-shaped areas outside of the apex of the affected area. The distinctness and conspicuous character of this ring make it evidently something more than an accidental feature. It can be traced through adjoining sections and shown to be a complete ring. Similar appearances in other eggs will be recalled, as, e. g., in that of the leech.

Turning to the nuclear phenomena at this stage, we see that the spindle lies close to the egg membrane. Centrosomes are inconspicuous, but a radiating arrangement of the alveoli may be made out at the poles. The spindle is in the equatorial plate stage (Fig. 22).

In another egg we find the chromosomes just separating (Fig. 23). There are eight pairs, and they are quite unequal in size, as was shown in the first polar spindle. They show a marked increase in size compared with those of the first division. The spindle here lies in a decidedly lighter staining area. The polar globule fills a bowl-like depression in the disc instead of a position at the side of the depression, as in the previous case shown. The eight dyads in the polar body are not fused together, and some retain a slightly dumb-bell like shape. The wall of the polar body is well marked. The polar ring was present in this, as in all of the other maturation stages obtained after impregnation of the egg.

The second maturation spindle is shown in Fig. 21 in a slightly earlier stage. The spindle is not yet completely reformed. One of the chromosomes lies between the equatorial plate and the egg membrane not yet being drawn into position. It has the appearance of a tetrad, in which case it would be a chromosome of the first division which failed to divide. It is, however, probably a dyad in the first stages of splitting. The chromosomes as shown above are originally pairs of dyads lying closely side by side. The polar globule is in this case more rounded than in the last, which is undoubtedly due to its not yet having had time to become flattened by the pressure of the egg membrane

One other stage of the second maturation spindle is shown in Figs. 9 and 24. In this egg the central core of the affected area directly underneath the spindle is different from the other cases obtained, being entirely free from deutoplasmic granules.

The egg nucleus is shown in Fig. 25 with the second polar globule just given off. The chromosomes are fused together into a mass with a somewhat crenate contour. The polar globule is still connected with the egg by a cytoplasmic neck, and its wall is not formed. The egg nucleus has penetrated the egg farther than is found to be the case in some later stages, but this may be explained by the exceptional fewness of yolk granules in the affected area which ordinarily might hinder its freedom of movement.

The further reconstructed egg nucleus is shown in Fig. 26. Both polar bodies are shown, being present in adjacent sections. The inner sphere and centrosome (?) is in this case recognizable and appears larger than during division. The egg nucleus does not have a distinct membrane.

Fertilization stages.—In a still later stage the egg nucleus is seen to be completely reconstructed and has a distinct membrane (Fig. 27). Yolk granules crowd about it so as to hide any other structures. The polar bodies were both found in the same section. The one which is farther

from the egg nucleus has the chromosomes nearly all fused together. The nearer one is a fused mass of chromatin with a ragged outline showing a decided tendency to form a network. In displaying this tendency it resembles its sister nucleus in the egg. It tends to enter a metabolic phase. The first polar body, on the other hand, retains the kinetic tendency like the second maturition spindle, although it fails to divide.

The polar ring is present at this stage, but has not been found later than this, being apparently obliterated by cytoplasmic movements occurring within the affected area.

The sperm nuclei which are found in this egg are rather faintly staining bodies and no one of them is especially near to the egg nucleus.

The next stage obtained shows the pronuclei very near together, one being slightly smaller and deeper in the egg (the male?) There is a hyaline area adjoining, but no distinct astral appearance (Fig. 10).

In a later stage the pronuclei, about the same size as in the last instance, are seen in contact (Fig. 11). A diagram (Diagram 1), shows the other sperm nuclei present and their distribution. Only one, and it a quite large one, is in the affected area, the rest being scattered away from the center in all directions.

At a later stage the conjugating nuclei are flattened against each other (Fig. 12). The cytoplasmic surroundings of the disc are shown in the drawing. The peculiar orientation of the lighter staining central area in the form of a cone which was seen in the maturation stages is not any longer manifested in any of the fertilization stages. There is a considerably vacuolated area at one side of the pronuclei and on the other side a curious apparently normal appearance like a hyaline channel extending from the vicinity of the nuclei toward the egg surface. Astral appearances if present are hidden by the yolk. The number of sperm nuclei shown in the diagram (Diagram 2), is only twelve, which is probably too small, as the series of sections was imperfect.

When the segmentation nucleus is formed the accompanying cytoplasmic changes in the germinal disc are so striking as to indicate plainly the approach of division (Fig. 13). The affected area is spread out laterally and shows a differentiation into a more hyaloplasmic margin and a granular interior. This fact is, however, still more clearly perceived by reference to a surface section as shown in the subsequent stage (Fig. 14). The segmentation nucleus (Fig. 13) lies near the center of the affected area, rather closely surrounded by yolk granules. It has moved nearer to the egg surface than at the time of copulation. There are no sperm nuclei remaining within the affected area, but a pair are to be seen a little distance outside of its margin. The segmentation nucleus may be

identified by its size and the appearance of the chromatin, as well as by its surroundings. It has a well-developed double contoured membrane. The contents are slightly plasmolyzed, a feature which has not been observed to occur at any earlier stage. The chromatin is beginning to be gathered into long threads.

The First Division.—A later stage containing the prophase of the first cleavage spindle is shown in Fig. 14, in horizontal section. The spindle lies at the center of a small area free from granules (Fig. 28). Centrosomes and asters are very indistinct, as would be expected at this stage of division. The centrosome is not a deeply staining granule. The cytoplasm shows only indistinct radiations. The spindle is rounded at the ends and rather broad. The chromosomes, sixteen in number, are partly in the equatorial plate. None of them are yet splitting. The prevailing shape of the chromosomes is that of a broad V. In the surrounding protoplasm may be seen the same appearances as in the stage of the segmentation nucleus (Fig. 14). There is an area of protoplasm whose outer border is hyaline and the center surrounding the nucleus is more granular. The area is elongated in the direction of nuclear division. Its margin shows an appearance like that of outpushings. These are large lobes, as if indicating an amœboid movement of the whole mass. Curiously enough, the granular interior conforms to the same outline, showing lobes corresponding to the outer margin. Balfour, **85**, states that "In elasmobranchs before segmentation commences, the germinal disc exhibits amœboid movements." Here these amœboid movements, if so the appearances described are to be interpreted, are seen to be confined to a region at the center of the germinal area whose diameter is about 0.5 mm., or about one-sixth the diameter of the inner area of the disc. The area of active protoplasm is differentiated into a more granular and a more hyaline pole. There are indications of a constriction which, if carried out, would thus divide the cytoplasm of the area qualitatively.

A stage of the first division is shown in Fig. 15, in which the nuclei are separated a considerable distance. They are of quite small size. The affected area of protoplasm shows a dumb-bell shaped figure and the nuclei lie at about the centers of the two ends. The hyaline outer border and the inner granular condition is still preserved. The first furrow is being formed at the constriction, but is shallow and does not appear in the section containing the nuclei. In the surface section it is seen as a broad, shallow depression filled with cytoplasm of a finely alveolar structure. Around the affected area lighter streaks may be seen extending out into the surrounding protoplasm. One blastomere is seen to be more hyaloplasmic than the other.

The nuclei and first furrow have been found at a stage when the nuclei are very much larger. They had moved apart relatively little compared with the former stage, while they had greatly increased in size. If the small size at the former stage indicated that little time had elapsed since division, then their movements at first must have been more rapid. Perhaps the cytoplasmic constriction would be the cause of the early, rapid separation of the nuclei. Whatever may be the link connecting nuclear with cell division, it would seem that the constriction of the cytoplasm must play a part in the separation of the nuclei. At this stage, the completion of the first division, there is no differentiated area about the nuclei recognizable. Apparently the amœboid changes cease during the resting stage of the nuclei.

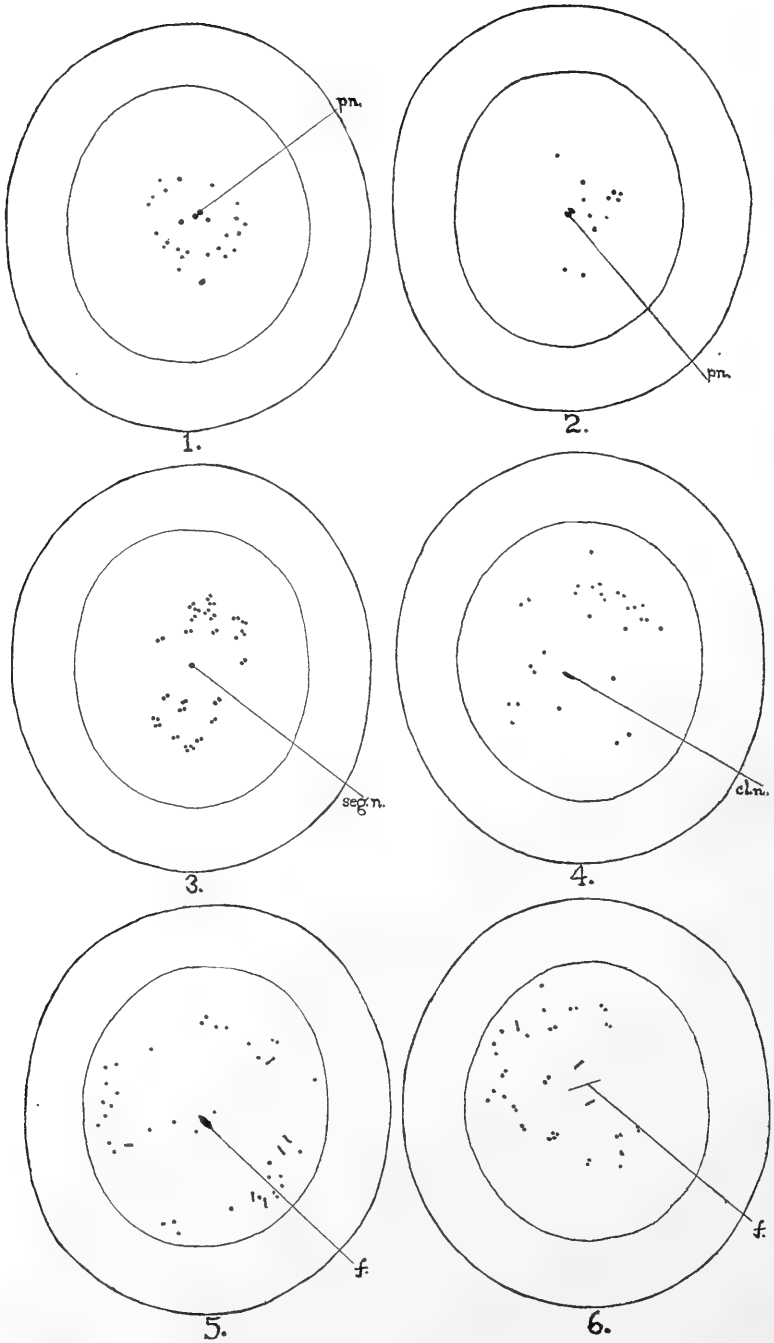
The Second Division.—In Fig. 16 is shown the beginning of the second division. The first furrow is longer. The nuclei are about equidistant from it. The spindles are formed and are in the prophase, approaching the equatorial plate stage. They lie in small areas free from granules. The differentiation in the cytoplasm is like that surrounding the first cleavage spindle, except for greater complexity. There is a clearly marked polarity. One blastomere is more granular, the other more hyaloplasmic. In the latter there is a complex affected area surrounded by homogeneous protoplasm. The hyaline border of the active area is even more distinct than at the first division. But this blastomere must apparently be identified with the more hyaloplasmic pole at the first division. The hyaline border shows a sinuous contour. The whole area is elongated, as before, in the plane of the next nuclear division. The prominences or outpushings are more complex. They correspond somewhat at the two poles, but are more developed at one pole (the left). There is an evident beginning of constriction, and division at this point would separate again a more hyaloplasmic blastomere from a more granular one. In the other blastomere the affected area has an even contour. One other egg was obtained at this stage and shows the same general features, but with minor differences in the apparent amœboid changes. It would hardly be expected that amœboid movements of this character would give rise to identical appearances in different eggs. The observation of Whitman, 87, on cytokinetic phenomena in general may be quoted in this connection: "They are diversiform in the extreme, rarely presenting regular form series, and thus stand in marked contrast with nuclear metamorphoses, which everywhere, both in plant and animal cells, exhibit a most remarkable uniformity."

Comparison of Cleavage and Maturation Divisions.—The appearances here described in connection with cleavage may be compared with the

maturation divisions. The body of active protoplasm is differently oriented in the two cases, as has been pointed out. During maturation the active protoplasm underlies the spindle and extends radially in the egg, widening centripetally, so that its appearance is roughly that of a cone from the apex of which the polar bodies are pinched off. At cleavage, the area assumes a horizontal position with reference to the surface, and a constriction occurs at its middle. At the first maturation division (Fig. 6a), the spindle lies at the center of a granular area encircled by a hyaline zone. From analogy with the cleavage divisions, it would appear that this hyaline zone is a normal cytoplasmic feature of the maturation division. Within it lie many sperm nuclei and it is the favorable zone of entrance for the male elements. It would thus appear to have a double function, the relations of which are not, however, necessarily close, since the entrance of spermatozoa is not confined to this zone. In the second maturation division the hyaline zone around the spindle seems less conspicuous than at the first.

Kupffer, **75** and **90**, pointed out that the primary differentiation of protoplasm seen in the unicellular organism, into an outer hyaline and an inner granular protoplasm surrounding the nucleus, is also found in the animal tissue cell and egg. In the egg of petromyzon, as shown by Böhm, to which Kupffer, **90**, made especial reference, this differentiation is clearly shown. The hyaloplasm, which during fecundation appears as a cap upon the egg, later moves back into the yolk and undergoes further amœboid changes, elongating in the direction of nuclear division. The behavior of the sphere substance in the egg of unio, as described by Lillie, **01**, may be compared with that of the active protoplasm in the pigeon. The sphere substance results from the growth of the egg centrosome and sphere, and extends across nearly the whole diameter of the egg, elongating in the plane of nuclear division. In the pigeon's egg one stage was found, of the partially reconstructed egg nucleus, where the centrosome and sphere appeared greatly enlarged, as described by Lillie (Fig. 26). Conklin's demonstration of protoplasmic currents in the egg of crepidula may be mentioned in this connection. As mentioned above Balfour states that in the selachian egg amœboid movements occur before cleavage begins. Whitman, **87**, insisted that the cytoplasm could not be regarded merely as a passive nutritive substance, although, as he said, the majority of writers are inclined to seek the *primum mobile* in the nucleus and to make the nucleus responsible for the kinetic phenomena displayed in the cytoplasm."

Loeb, **95**, relying upon Quincke's experiments and certain experiments of his own upon the echinoderm egg, ascribes cell-division to diffusion



DIAGRAMS 1-6. NUMBER AND DISTRIBUTION OF ACCESSORY NUCLEI IN EARLY STAGES OF PIGEON'S EGG.

EXPLANATION OF DIAGRAMS 1-6.

DIAGRAM 1. Diagram showing number and relative position of accessory nuclei in the egg. The male and female pronuclei are in the center, lying in contact. Two sperm nuclei nearer than the rest. Same egg as in Fig. 11. × 10.

DIAGRAM 2. Pronuclei in closer contact. Same egg as shown in Fig. 12. Owing to the incompleteness of the series the number of sperm nuclei is too small. × 10.

DIAGRAM 3. Stage of segmentation nucleus. Accessory nuclei are mostly in pairs, indicating previous division. Same egg as Fig. 13. × 10.

DIAGRAM 4. Stage of first cleavage spindle. Same egg as Fig. 14. × 10.

DIAGRAM 5. First furrow is beginning to appear at surface (f). The two cleavage nuclei equidistant. Same egg as in Fig. 15. × 10.

DIAGRAM 6. Beginning of second division. The accessory nuclei appear nearer the center of the disc than in the previous stage. × 10.

phenomena and amœboid movements occurring on the surface of the egg along the circle whose plane separates the two astral systems.

The Early Cleavages.—In Figs. 40-45 are shown the 2-4-8-16 cell stages, drawn from a surface view. The accessory cleavage is also shown, except in the sixteen-cell stage. The first furrow crosses the disc along its shorter diameter. It is slightly eccentric in position. In the four-cell stage is seen the so-called " cross furrow " connecting the second furrows. In the eight-cell stage, considerable variety exists in the position of the furrows. A more regular type is shown in Fig. 43, and an irregular one in Fig. 42.

The more regular type shows meridional furrows at quite corresponding positions in the four quadrants. In the sixteen-cell stage shown the accessory cleavage is not represented (Fig. 45). The nuclei in the anaphase of division are shown as they appeared in a surface view in a whole mount of the blastoderm, and give an idea of the relative size of nuclei and blastomeres.

In the sixteen-cell stage there is a clearly marked polarity of the egg due to the small size of the blastomeres on one side. This asymmetry of cleavage was pointed out by Kölliker in the case of the chick as producing an evident polarity during the early cleavages, whose relation, however, to the polarity of the embryo is undetermined.

Development of the Accessory Nuclei.

The accessory nuclei, whose appearance soon after the time of entrance was described in connection with Fig. 6b, have been found in later stages, varying considerably in number. In fertilization stages from twelve to twenty-five have been counted. After division sets in among them their number in some cases becomes very great, and no attempt has been made to count them. The diagrams (Diagrams 1-6) show the number and distribution of these nuclei in a series of stages. The general fact is disclosed that they migrate away from the point of entrance and soon become outside of the vicinity of the pronuclei. During the earlier stages of copulation one or more of the accessory nuclei may remain in the vicinity within the affected area of the germinal disc. But this is not true of the later stages, as is indicated by the absence of accessory nuclei in Figs. 14-16.

In Fig. 13 a single pair are found at one side, near the affected area. This pair are in close apposition, as if conjugating. Moreover, twenty-five pairs of such nuclei are found in this egg together with some earlier division stages. Some of the pairs are in apposition, but most of them are a slight distance apart, some being in a stage very soon after division.

A difficulty is presented in finding the division stages, on account of the surrounding yolk. It must be remembered that these nuclei are not in the favorable region for observation in the center of the disc where the yolk granules are fewer, but they are migrating through the deeply staining surrounding region. Hence during the division stages when at their minimum size they are to be seen only under the most favorable circumstances. Several clumps of chromatin threads in the spireme and later stages were seen, enough to indicate the probable presence of other division stages. The resting nuclei are, of course, larger and easily seen. For the above reason the details of the first division of the nuclei have not been made out in the very limited material at hand. This difficulty, however, later disappears, and the mitotic division of these nuclei may be made out with perfect ease.

If the sperm nuclei divide before the cleavage nucleus, then the rate of division of the latter is a resultant of a slower and a faster rate. The rate of division of the unfertilized egg nucleus may be considered as approaching zero. There have been contradictory observations as to whether the unfertilized blastodisc of the chick may segment parthenogenetically. Appearances have been observed which at any rate suggest this. Barfurth, **94**, offers a different explanation of these phenomena holding that there is no true cleavage in the unfertilized eggs. Assuming that possibly it may occasionally happen that the unfertilized egg nucleus may divide for several generations of cells, it is in accord with the accepted view as to the nature of the sperm protoplasm that the sperm nucleus should show a faster rate of division than the egg nucleus, and that the fusion nucleus should have a somewhat slower rate than that of the sperm nuclei. But Rückert found in the selachian egg that the sperm nuclei divide synchronously or nearly so, with the cleavage nucleus. Oppel, **92**, found in the reptilia, on the other hand, that the accessory nuclei divided more slowly or not at all in many cases. It is thus seen that special adaptations have arisen in different groups. Environment seems to have more to do with the division of the sperm nuclei than the nature of their own protoplasm.

In the course of their further migration the nuclei reach the coarser yolk surrounding the inner zone of the germinal disc. Here, either because of a difference in the chemical nature of the materials surrounding them, or because their progress is impeded by the coarser yolk granules, the nuclei remain and their division is followed by a cleavage of the surrounding cytoplasm. The first indications of this accessory cleavage on the surface of the egg are seen when the first furrow is established between the cleavage nuclei (Fig. 40). The continuation of this cleavage and division of the surface into small cell-like areas is indicated in the

two-, four- and eight-cell stages. In the later stages obtained the accessory cleavage is not shown in the figures. This accessory cleavage is then set up after the second mitotic division of the sperm nuclei. They are found at the time of this division surrounded by wide cytoplasmic areas free from yolk granules. The resting nuclei after the first division are accompanied by small areas of a "sphere substance" which entirely resembles this material. This sphere substance rather than being regarded as an organ accompanying the nucleus would seem to be an accumulation of the products of the nuclear activity. During the migration of the nuclei, the amount accumulated next to a nucleus appears small (Fig. 36), but after they are settled down the substance soon gathers in large quantities. Of course this statement is not meant to imply that the sphere substance may not at certain times take on a definite form, like a permanent organ, as in the young ova, for instance. According to the view of Van Bambeke, 97, the sphere substance is the center of formation of plastic and of nutritive elements.

To explain the peripheral migration of the sperm nuclei, Rückert has developed a theory of mutual repulsion which applies to all nuclei of a like character, and is exerted by and through the means of the sphere substance. The sperm nuclei show a mutual repulsion for each other, which prevents their conjugation with one another. The cleavage nuclei have a superior power of repulsion, and so drive the sperm nuclei from the cleavage area into the yolk. The egg nucleus having no centrosome and sphere, or only a slightly developed one, is on the contrary attracted to the male pronucleus. The early and rapid migration of the sperm nuclei out of the cleavage area is, however, a fact which does not seem to fall within this explanation. The sperm nuclei for some reason migrate to the periphery and give rise to an accessory cleavage there, while the egg is still in the two-cell stage. Only a few straggling nuclei are at this time remaining within the inner area of the disc. There seems to be a tendency on the part of the sperm nuclei to migrate, only one of them being caught at the early stage by the attraction of the female nucleus; and this conclusion is certainly not inconsistent with the motility of the sperms during the stage of their free existence. As an active cause for the migration of the sperm nuclei, it might be assumed that the activity is but the continued expression of the labile nature of the protoplasm which gives the sperm its motile character during the period of its independent existence. An indication of the rapid movement of the sperm nuclei has already been pointed out, namely, that the accumulation of altered protoplasm or "sphere substance" about the nuclei while migrating in the germinal disc is very small. As soon,

however, as they reach the marginal yolk they become surrounded by wide areas of protoplasm free from granules of yolk, owing, according to the assumption, to the cessation of their rapid movements and their delimitation to fixed areas, which results in the accumulation about them of the products of their metabolism, instead of its diffusion into the surrounding protoplasm.

Mitosis in the Sperm Nuclei.—The mitosis of the accessory nuclei appears to be normal in the early divisions, at least in the sense that it results in an equal division of the chromosomes. The details of mitosis have not been compared with that of the cleavage nuclei, although such a study might indeed be valuable. The determination of the number of chromosomes in the spindle has a bearing upon the origin of the nuclei, of course. It is not asserted, since it has not been definitely proved, that mitosis is always perfectly normal, even at this stage, since abnormalities do appear later which lead eventually to amitosis. No pluripolar spindles have, however, as yet been observed. Regular equatorial plate stages are found. The reduced number of chromosomes is present, which is eight. In the metabolic nuclei the chromatin network is somewhat finer than that of the cleavage nuclei. This difference extends to the fully formed chromosomes, which are narrower and somewhat more elongated than those of the cleavage nuclei. In the prophases very long, slender chromosomes are formed which become shorter and thicker as they approach the equatorial plate stage. A typical longitudinal splitting of V-shaped chromosomes takes place (Fig. 33), and as the daughter chromosomes pass to the poles, one end of each becomes thicker. Gradually the chromatin accumulates at this end (Fig. 34) until in the late anaphase the chromosomes appear as short oval bodies approaching a spherical shape (Fig. 35). The achromatic structures are well defined. The centrosome is a sharply defined, deeply staining spherical granule, not so large as in the late cleavage nuclei, but decidedly more conspicuous than the centrosome of the maturation and early cleavage stages. The spindle fibers are distinct and the spindles are very regular in form. These characteristics of mitosis and their similarity to that found in later cleavage stages and dissimilarity with that found in the early cleavage seems clearly correlated with the nature of the substance by which the nuclei are surrounded, which is in the one case a highly plastic cytoplasm, the immediate product of the nuclear activity, and in the other is the largely unmodified egg cytoplasm, which from its coarse alveolar structure reacts differently to the mitotic forces and gives less evidence of their operation by a change in form than does the more plastic medium.

In regard to the synchronousness of division in the sperm nuclei, there appears to be a considerable difference. In one egg of the two-celled stage, over one hundred sperm nuclei were present. The accessory cleavage began at one side and here the nuclei had nearly all passed into a resting stage. On the opposite side of the disc, the nuclei were nearly all in some phase of division from spireme to late anaphase. It did not seem that concentric zones could be distinguished, as Rückert has found to be the case in the selachian, in which the gradations in phase of division could be found in successive zones. Rather in this egg there was a difference in phase on opposite sides, or what may be called a polar difference. The evidence of this is seen also in the beginning of the accessory cleavage, as shown in Fig. 40.

The Yolk Nuclei of Later Cleavage Stages.

With the advance of the cleavage nuclei, the sperm nuclei are driven into the surrounding yolk. In a stage about fifteen hours after fertilization, the sperm nuclei were found dividing amitotically. The intervening stages have not been filled in. The identity of the yolk nuclei at this stage with the earlier sperm nuclei is undoubted in the light of the selachian egg, whose phenomena can be duplicated, at least as to chief details, in the pigeon.

Balfour has described the yolk nuclei in the chick as lying at the margins of the blastoderm, and under the peripheral cells, but not under the center. The nuclei are found very largely in nests or clusters, the members of which are very unequal in size. Sometimes 6-8 nuclei may be found thus clustered together (Figs. 39a, b, etc.). They are surrounded by wide areas of protoplasm free from granules of yolk. Besides the nuclear nests, many are found singly and these very frequently at the margin of the blastoderm near the surface. These often show a distinct difference in staining capacity, retaining the stain with more tenacity than the underlying ones. This difference would seem to be correlated with the environment, since these are found in the coarse, deeply-staining, yellow yolk and the underlying ones in the white yolk. There are also "giant" resting nuclei as large as the entire protoplasmic area which surrounds one of the nuclear clusters (Fig. 37b).

Transitional stages from mitotic to amitotic division may be found at this period. In some of the protoplasmic areas are found nests of daughter nuclei not yet reconstructed (Fig. 38). The separate chromosomes or chromatin vesicles are distinct or partially fused together. In some of the groups of chromatin vesicles approximately eight could be counted, although the exact number could not be identified on account

of fusion, and also apparent disintegration of some. These appearances may arise from pluripolar spindles, which have not yet been found in the pigeon, but which are undoubtedly to be found here as in the selachian. Other evidences of attempts at mitosis are to be found. Often a spireme is found of irregular appearance, indirect division proceeding no farther. There were no accessory cleavage furrows recognized at this stage.

Are the nuclei incorporated into the cleavage area? There is no affirmative evidence on this point. On the contrary, there is a distinct separation between cleavage cells and yolk underneath the blastoderm, the marginal cells having complete cell boundaries. No nuclei at all resembling the yolk nuclei have been found within the cleavage area. The cleavage nuclei are distinct in appearance and are not easily to be confused with the nuclei in the yolk.

The only evidence obtained having a possible bearing on the fate of the yolk nuclei is the occurrence of great numbers of peculiar refractive bodies closely associated with the nuclei in the large nuclear nests. These bodies resemble the disintegrating nucleoli of the late ovarian egg. In a nest such as shown in Fig. 38 there are large masses of this refractive substance made up of clusters of vesicles. There are also isolated rod-like bodies of the same material. May these not indicate that there is a constant reduction in the amount of chromatin material due to "karyolitic" action? The yolk nuclei are very numerous at this stage, and form a fringe around the blastoderm, but do not go far out into the yolk. Their numbers at the start, if augmented by division followed by migration, ought soon to fill the yolk, as it would seem. On the contrary, the margin of the blastoderm seems to be the only region occupied by them. The liquefied products of this karyolitic action are doubtless absorbed by the embryonic cells. The refractive bodies are probably material in process of dissolution, as is apparent in the case of the nucleoli of the late ovarian egg.

Polyspermy in Other Eggs.

The term physiological polyspermy has been applied to all cases where more than one spermatozoon normally enters the egg. The fate of the supernumerary sperms is by no means the same in the different groups in which the phenomena occur. Hitherto, in the elasmobranchs alone has their persistence to form yolk nuclei or "merocytes" been observed. The nearest approach to this condition was found by Oppel, 92, in the reptilia, where the sperm nuclei though present in large numbers, divided slowly and karyolitically, and soon degenerated. The evidence in the case of the reptilia is, however, fragmentary.

If the cause assigned by Rückert for the occurrence of physiological polyspermy be correct, namely, the absence of protection against it on account of the thinness of the egg covering in these internally fertilized eggs, it might well be expected to occur in the bird's egg also. Balfour, 85, stated in regard to the chick, that "In the bed of white yolk nuclei are present which are of the same character and have the same general fate as in Elasmobranchs. They are generally more numerous in the neighborhood of the thickened periphery of the blastoderm than elsewhere."

Among the amphibia, polyspermy has been found in the urodela. Thus Jordan, 93, found it to be universal in the newt. He states that "there is every reason for regarding such physiological polyspermy in the newt as a natural, normal and in fact usual occurrence." The extra nuclei degenerated shortly after the fusion of the pronuclei. Fick, 92-93, found polyspermy occurring in axolotl, but inconstant. Braus, 95, in triton found the sperm nuclei dividing amitotically from the start, a fact which Rückert correlates with the entrance of the spermatozoa through the yolk, since in elasmobranchs the sperms enter through the germinal disc and change from the mitotic to the amitotic method of division after they have migrated into the yolk. The anura, on the other hand, are monospermic according to the evidence of Hertwig, Born, 86, Roux, 81, and King, 01, although opposing observations were recorded by Kupffer, 82, in the case of Bufo.

In the elasmobranchs the yolk nuclei have been the subject for much controversy and speculation. Balfour, 74, recognized the existence of such nuclei in the late cleavage stages of the selachian blastoderm. He surmised that they arose spontaneously in the yolk. Schultze, 77, disagreed with such an assumption as to their origin and took it for granted that they arose from the cleavage nuclei. Rückert, 85, traced these free nuclei as far back in development as the eight-cell stage, which was the earliest stage he found. He argued that they arose from an equatorial cleavage of the nuclei of the four-cell stage, and that their position in the yolk indicated that they were the homologues of the nuclei of the vegetative pole in the frog's egg. Their peripheral position was taken as strongly favoring such an homology. Rückert termed them "merocytes," indicating thereby that they were parts of cells, namely, the nucleus with some surrounding protoplasm, which after division migrated away from the cellular region into the yolk. Kastschenko, 88, found these merocytes in the stage of the formation of the first furrow. He proposed a theory that the first cleavage nucleus gave rise to a multinucleate plasmodium before the first division of the egg. Rückert, 90-92,

pursued the investigations still further, and by the discovery of fertilization stages, was enabled to announce the origin of the much-discussed yolk nuclei from spermatozoa. He thus not only accounted for the origin of the merocytes, but established the fact of physiological polyspermy for the selachian group. He traced a continuous series of stages from the entering sperm head to the fully developed merocyte. He obtained finally the conclusive evidence of their origin from spermatozoa by determining that the dividing nuclei contained only one-half the somatic number of chromosomes. Rückert's results for selachians were confirmed by Samassa, **95**, Beard, **96**, Sobotta, **96**. His own complete account appeared in 1899.

The present state of the controversy which involves the ultimate fate of the merocytes is outside of the province of this paper. The question whether there may be another generation of yolk nuclei arising in late cleavage stages, homologous with the periblast of teleosts, has been the subject of controversy chiefly between His and Rückert.

The announcement of Rückert of the origin of merocytes from spermatozoa necessitated the modification of the prevalent assumption as to the universally pathological nature of polyspermy and opened up a field of inquiry as to the causes of normal polyspermy; its adaptiveness; its difference from the so-called pathological type; the influences which prevent multiple conjugation with the egg nucleus; the cause of the migration of the supernumerary sperms into the yolk; their change from mitotic to amitotic division, etc. The identification of these sperm nuclei with the long known "yolk nuclei," to which had been assigned by common assumption the function of yolk digestion for the embryo, both in selachians and teleosts, raised the question whether in reality in the selachians the sperm nuclei have a normal or physiological role in embryonic development.

As mentioned above, the presence of nuclei in the yolk during the early cleavage stages, forming a syncytium supposedly derived from the cleavage nuclei, had been used as an argument to support the theory of the homology of the yolk of the selachian egg with the lower pole cells of the frog's egg.

Rückert holds that the cause of polyspermy in the selachian egg is simply the absence of protection against it, due to the thinness of the egg membrane. The phenomena of conjugation of sperm nuclei with each other and their multiple conjugation with the egg nucleus, seen in the case of nicotinized eggs (Hertwig, **87**), he holds to be due not to polyspermy, *per se*, but to changes brought about by nicotinization. Such phenomena are absent in polyspermatic eggs, and he proposes a theory that

the sperm nuclei exhibit normally a repulsion for each other due to the presence and activity in some way of their accompanying sphere substance. The absence or feebler development of the sphere substance in the egg nucleus accounts for the mutual attraction displayed by the male and female pronuclei. Moreover, the mutual repulsion of sperm nuclei disappears with the change of their environment from the germinal disc to the yolk as it disappears in the abnormal environment of nicotinized eggs. Hence conjugation of nuclei occurs in the yolk, giving rise to giant nuclei, pluripolar spindles and progressively increasing irregularity of division ending in amitosis.

As to the adaptation of polyspermy to the large meroblastic egg, Boveri suggested that polyspermy was necessary in the case of the large egg to insure certainty of fertilization. Rückert maintains that the force of this argument is weakened by the fact that the region where the sperms may enter is very limited and is in close proximity to the egg nucleus. Sobotta accepted the above view of Boveri and also argued that the size of the egg was the factor which prevented multiple conjugation with the egg nucleus, since the sperms could never in so large an egg enter at exactly the same time. Hence they would always be unequal in development, and the largest would become the male pronucleus. Rückert points out the inconsistency of maintaining that the size of the egg requires the entrance of many sperms to insure fertilization, and that the size of the egg is also what prevents multiple fertilization. Rückert believes that the mutual repulsion of sperm nuclei is at least to be regarded as a fact in the selachian egg, if not proved true for all monospermic eggs. He thinks it improbable that the sperm-nuclei have a normal function in the embryonic development, and leaves open the question of a possible second generation of nuclei arising from the cleavage cells in later stages homologous with the periblast of teleosts.

As to the cause of migration of the accessory nuclei into the yolk, Rückert adduces his theory of repulsion, holding that the sperm nuclei are driven from the germinal disc by the advancing cleavage nuclei, owing to their superior power of repulsion. If the observations upon the pigeon in this regard prove anything, it is that the sperm nuclei migrate so early to the periphery of the germinal disc that it is difficult to believe they do this under the influence of the cleavage nuclei. As pointed out, they are found in the accessory cleavage region, far removed from the affected area in the center of the disc, which seems to be the sphere of influence of the cleavage nuclei, as early as the formation of the first cleavage furrow. Moreover, they are nearly absent from the intermediate region at this time. This seems to point to the independent

activity of the sperm nuclei, rather than any mechanical driving of them from the inner region. What chemotactic influences there may be present we of course have no means of knowing.

Some Features of Mitosis in the Pigeon's Egg.

Centrosomes and asters are structures which are frequently asserted to be absent from eggs heavily laden with yolk. For example, in the maturation stages of some amphibians they are said to be wanting. Speaking of the egg of unio, Lillie says that "rays form more readily in protoplasm free from yolk granules." The bird's egg is certainly as heavily laden with deutoplasmic granules as any, and these granules are of relatively large size.

This question cannot be considered properly without taking into view more than the earliest phases of development. If we take these into view in connection with the later cleavage, we find that there is a progressive increase in the distinctness of achromatic structures as development proceeds. A typical mitosis from a rather late cleavage stage is shown in Fig. 29. Here the centrosome is a very large, well-defined granule, and spindle and astral fibers are distinct.

In inquiring into the reason for the feeble development of astral fibers in the maturation stages of the pigeon's egg, it does not seem that the interference of yolk granules in all cases accounts for the fact. For occasionally yolk granules are not especially near to the spindle, and the structures in question are not exceptionally well developed in these cases. In the maturation stages in the pigeon's egg, the spindle is sometimes in an area free from granules, but the achromatic structures are essentially similar in all cases. Centrosomes and asters are inconspicuous, but the alveolar structure about the poles of the spindle, when copied with the camera, shows a somewhat regular radiate arrangement. There is no well defined centrosome, more than perhaps a cluster of minute granules difficult to make out.

Some light is thrown on this matter of asters and centrosomes by mitosis in the sperm nuclei. As has been pointed out, these nuclei when they reach the periphery of the disc in their migrations, come to rest and become surrounded by large areas of cytoplasm, which is identical in appearance with the sphere substance associated with the nuclei earlier. As has been suggested, this cytoplasm is apparently the product of the activity of these nuclei, and is evidently of a highly plastic nature, giving rise in division to very regular mitotic figures, and well defined centrosomes.

In the later cleavage stages of the blastoderm, the same is true. The

nuclei then are surrounded by entirely similar areas. They are at this stage limited in their movements by the cell boundaries, and so confined to very small areas. Consequently they become surrounded by cytoplasm, which is the product of their own activity in altering the constituents of the yolk. This material is highly plastic and responds to the forces operating in mitosis so as to produce regular figures.

Compare with the limited movements of these nuclei the wide migrations of the nuclei resulting from the first cleavage, and we see that the latter have no chance to become surrounded by an altered material, since all products of nuclear activity must rapidly become diffused into the surrounding cytoplasm. The reason for the different appearance in mitosis is seen when we compare the alveolar structure of the unaltered egg cytoplasm with that surrounding the later cleavage nuclei. The criginal egg cytoplasm is coarser, i. e., the alveoli are larger, and takes the cytoplasmic stains much less deeply. This is not very apparent in the drawings. In the maturation stages the absence of a metabolic phase of the nucleus for so long a period makes the surroundings of the nucleus least favorable of all apparently for the production of typically regular figures. It has been noted by some observers that the second maturation division differs from the first in the poorer development of asters, a phenomenon which might be due to the altered character of the surrounding cytoplasm. It would seem in the case of the pigeon's egg that the hyaline zone surrounding the first polar spindle (Fig. 6a) is not reformed so conspicuously at the second division, there being only scattered vacuoles present at this period in the region surrounding the nucleus. The suggestion that the deutoplasmic granules surrounding the nucleus in these early stages inhibit the formation of achromatic structures is perhaps an incomplete explanation, since the nature of the cytoplasmic groundwork may be a more fundamental cause.

Conclusions.

1. As a result of the monogamous habit of pigeons, ovulation is normally held in abeyance till aroused by the stimulus received from the male. The passivity of the female is compensated by the highly developed and complex instincts of the male bird. The determination of the time of fertilization and egg-laying must date from the time of mating. The second egg of a pair is set free from the ovary and enters the oviduct within a few hours after the first is laid. The egg is impregnated before entering the oviduct.

2. Polyspermy is normal. The most favorable region for entrance of sperms is the "fovea," in a zone surrounding the egg nucleus. Never

more than one male nucleus has been found in very close proximity to the egg nucleus.

3. The stage of development of the egg may be approximately inferred from its position in the oviduct. The first polar spindle is formed in the ovarian egg. The first cleavage occurs about the time the egg is entering the shell-gland. The time elapsing between impregnation and the first cleavage is apparently between two and three hours.

4. The polar bodies lie within the egg membrane, in a depression in the cytoplasm. The second disintegrates before the first, showing a tendency to form a network and become metabolic like the egg nucleus.

5. There is an area of active protoplasm surrounding the nucleus which during the maturation stages is oriented as a cone with the spindle at its apex, from which the polar bodies are pinched off. In preparation for cleavage, this area becomes oriented horizontally in the germinal disc. It undergoes amœboid changes and displays a differentiation into an outer hyaloplasmic and an inner granular area. It elongates in the direction of nuclear division, and divides with the division of the nucleus. The appearance of amœboid movements dies out during the resting period of the nucleus, and reappears at the second division. One blastomere is more hyaloplasmic than the other, and shows more complex amœboid changes.

6. The supernumerary sperms which enter the egg pass from the point of entrance toward the periphery of the disc. The accessory nuclei undergo division earlier than the cleavage nucleus. At the margin of the inner disc they come to rest within the coarser granular material, and give rise to an accessory cleavage on the surface of the disc. They divide mitotically without abnormalities, so far as discovered at this stage. They contain the reduced number of chromosomes, which is eight. The chromosomes differ in shape from those of the cleavage nuclei and the maturation spindles, being more slender. In late cleavage these nuclei are found outside the blastoderm, at or near the margins, and dividing amitotically. Some traces of abnormal mitosis were found.

7. Asters and centrosomes were found in the maturation stages, though not conspicuously developed. There is a progressive increase in the distinctness of these structures as the nuclei become limited to narrower areas by cell division, so as to become surrounded by the more plastic cytoplasm resulting from their activity in altering the yolk. The less pronounced development of these structures in the maturation and early cleavage stages seems due to the nature of the cytoplasmic groundwork, as well as to the casual interference of yolk granules. The sperm nuclei likewise do not display well developed achromatic structures till they are

delimited within narrow boundaries in the accessory cleavage area, when they become surrounded by large cytoplasmic areas free from yolk granules and display well developed and regular mitotic figures.

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EXPLANATION OF PLATES.

Plate I.

Fig. 1. Ovarian egg 1.4 mm. in diameter, showing nucleus. × 120.

Figs. 1a and b. Nuclei of ovarian eggs about 1 mm. in diameter. × 312.

Fig. 2a. Nucleus of ovarian egg 4 mm. in diameter. × 120.

Fig. 2b. Horizontal section through germinal disc of ovarian egg, ½ inch in diameter, showing nucleus; *a*, group of chromosomes; *b*, chromatin network. × 120.

Fig. 3. Nucleus of an ovarian egg ⅝ of an inch in diameter. × 120.

Fig. 4a. Vertical section through germinal disc and nucleus of ovarian egg, ¾ of an inch in diameter. × 50.

Fig. 4b. Nucleus of same, enlarged; *a*, group of chromosomes; *b*, group of nucleoli; *c*, refractive substance; *d*, wall of nucleus; *e*, follicular envelope of egg, outer layers of capsular wall not shown. Combination of two sections. × 385.

Fig. 5. Vertical section through disc of mature ovarian egg, taken from ovary after laying of first egg. Time, 7.30 P.M.; *a*, spindle; *B*, perivitelline layer; *c*, layer of substance free from deutoplasmic granules. × 200.

Plate II.

Fig. 6. Surface appearance of germinal disc about time of fertilization; *a*, inner area; *b*, outer zone; *c*, fovea. × 10.

Fig. 6a. Horizontal section of germinal disc of egg loosed from ovary and not yet entered oviduct. Time, 9.00 P.M.; *a*, egg nucleus; *b*, sperm nuclei; *c*, polar ring. × 125.

Fig. 6b. Same enlarged, showing zone in which the sperm nuclei lie. × 1000.

Figs. 7a-h. Stages in transformation of entering sperms. × 2000.

Fig. 7i. Inwandering follicular cell. × 2000.

Fig. 8. Vertical section through disc showing second polar spindle, polar body and surroundings. Combination of two sections. Position in upper end of oviduct. Time, 7.45 P.M. × 200.

Fig. 9. Vertical section, showing second polar spindle, polar body and surroundings. Time, 10.15 P.M. × 333.

Fig. 10. Vertical section. Pronuclei and surroundings. ♂ pron. at left. From two sections. Time, 11.50 P. M. × 400.

Fig. 11. Vert. section, showing pronuclei. Time, 10.15 P. M. × 400.

Fig. 12. Vert. section, showing pronuclei and surroundings. Time, 10.40 P. M. × 200.

Fig. 13. Vert. section, showing segmentation nucleus and surroundings. Pair of sperm nuclei at left. Time, 12 P. M. × 200.

Fig. 14. Horizontal section, showing first cleavage spindle and surroundings. Position in oviduct at constriction between upper portion and shell gland. Time, 10.30 P. M. × 200.

Fig. 15. Horizontal section, showing first pair of cleavage nuclei and surroundings. Combination of two sections. Time, 12 P. M. × 80.

Fig. 16. Horizontal section showing first pair of cleavage nuclei at beginning of second division and surroundings. *n*, nucleus; *f*, first furrow. Position in shell gland of oviduct. Time, 1.00 A. M. × 80.

Plate III.

Fig. 17. Horizontal section through nucleus of ovarian egg (Fig. 2b), showing group of chromosomes. *a*, pair of dyads; *b*, refractive substance in vesicular mass. × 2000.

Fig. 18. Group of chromosomes in equatorial plate from a mature ovarian egg. Same stage as Fig. 5, but from a different egg. × 2000.

Fig. 19. Group of chromosomes in equatorial plate of first polar spindle, showing central spindle granules. From same egg as Fig. 6a. × 2000.

Fig. 20. Vertical section, showing first polar spindle. Central spindle granules present as in 19, but not drawn. Egg clasped by funnel of oviduct. Time, 8.50 P. M. × 2000.

Fig. 21. Vert. section, showing second polar spindle, not completely formed and first polar body. One chromosome not in equatorial plate. Combination of two sections. Time, 8.55 P. M. × 2000.

Fig. 22. Vert. section, showing second polar spindle and first polar body. See Fig. 8. × 2000.

Fig. 23. Vert. section showing second polar spindle and first polar body. Chromosomes are separating. Combination of two sections. Time 8.15 P. M., × 2000.

Fig. 24. Second polar spindle. See Fig. 9.

Fig. 25. Vert. section, showing polar body, and egg nucleus as a fused mass of chromosomes. Combination of two sections. Time, 10.45 P. M. × 2000.

Fig. 26. Vert. section, showing polar bodies, egg nucleus without definite membrane, inner sphere enlarged. Combination of two sections. Time, 8.55 P. M. × 2000.

Fig. 27. Vert. section, showing completely reconstructed egg nucleus and polar bodies. Time, 8.30 P. M. × 2000.

Fig. 28. Horizontal section showing first cleavage spindle in prophase. Chromosomes not drawn into equatorial plate. See Fig. 14. × 2000.

Fig. 29. Spindle from cell of blastoderm about fifteen hours after fertilization. Chromosomes not all shown. × 2000.

Fig. 30. Sperm nucleus from 2-cell stage of the egg. In prophase of division. × 2000.

PLATE IV.

FIG. 31. Pair of sperm daughter nuclei. See Fig. 13 at left. × 1000.

FIG. 32. Sperm nucleus in prophase of division. × 2000.

FIG. 33. Sperm nucleus in division; shows chromosomes splitting. A vesicular refractive body and a yolk granule at right. × 2000.

FIG. 34. Anaphase of division of sperm nucleus in polar view, lighter group of chromosomes are in different plane. × 2000.

FIG. 35. Late anaphase of division of sperm nucleus. Figures 30-35 are all from the same egg. × 2000.

FIG. 36. Vert. section through blastoderm about fifteen hours after fertilization. Shows nuclei free in the yolk at the edge of the blastoderm. One nuclear nest is shown. × 120.

FIG. 37. Similar to above. A "giant nucleus" is present in yolk. × 120.

FIG. 38. From same egg as above. Shows an area in which two groups of chromatic staining bodies lie. *a*, chromatin vesicle; *b*, refractive body; *c*, yolk. × 2000.

FIGS. 39 a, b, c, d, e. Nuclei in yolk showing amitosis. × 1000.

FIG. 40. Surface view of germinal disc showing first furrow and accessory cleavage beginning at one side. Time, 12.20 A. M. × 10.

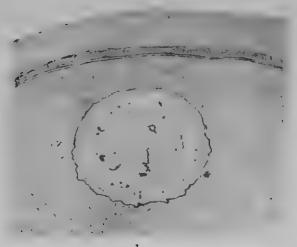
FIG. 41. Surface view of four-cell stage, showing accessory cleavage. Time, 3.15 A. M. × 10.

FIG. 42. Surface view of 8-cell stage with accessory cleavage. Time, 2.10 A. M. × 10.

FIG. 43. Surface view of eight-cell stage. Accessory cleavage not shown. Time, 3.55 A. M. × 10.

FIG. 44. Surface view of sixteen-cell stage. Accessory cleavage not drawn. × 10.

FIG. 45. Surface view of sixteen-cell stage. Daughter nuclei are visible in a whole mount of the disc and are shown in the drawing. Accessory cleavage not drawn. The boundary represents the margin of the inner zone of the germinal disc. × 20.



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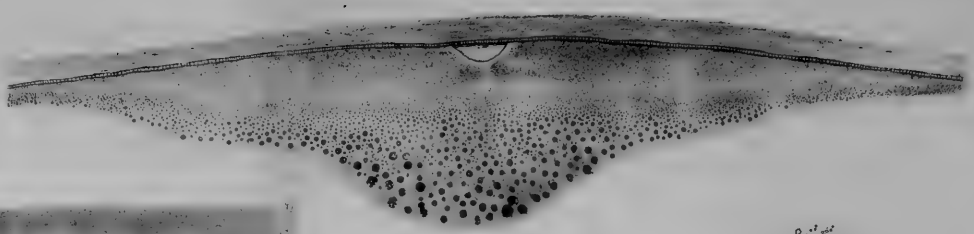

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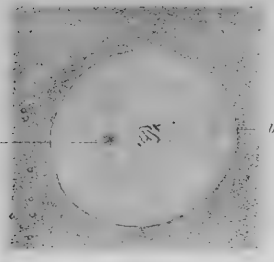

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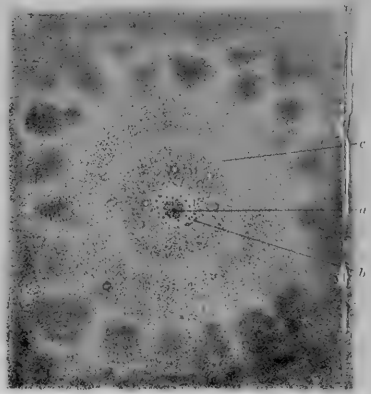

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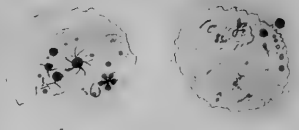
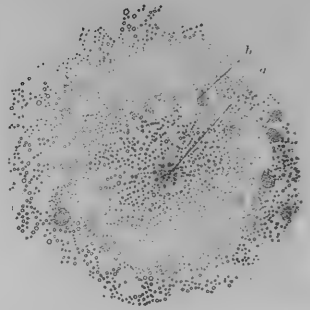


1 *a* 1 *b*



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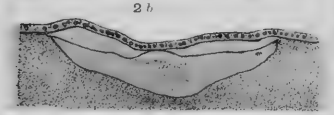
2 *a*



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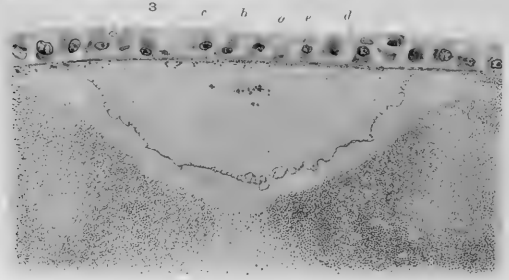

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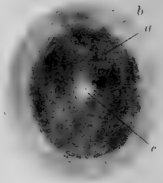

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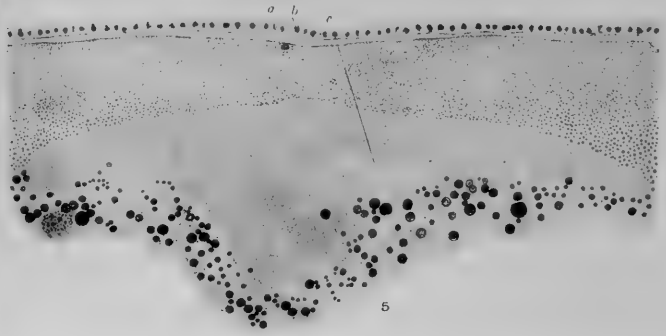

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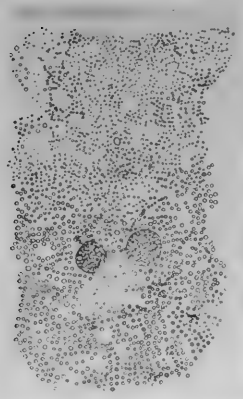

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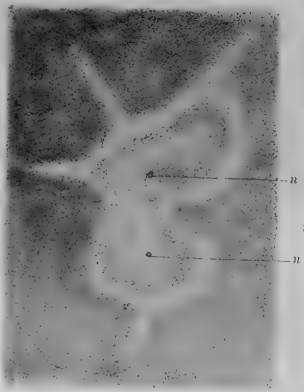

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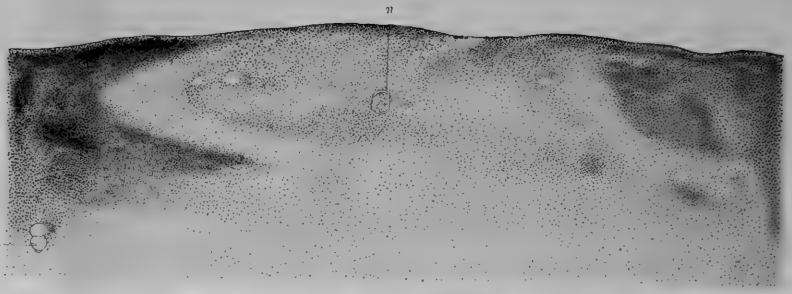

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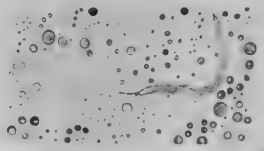

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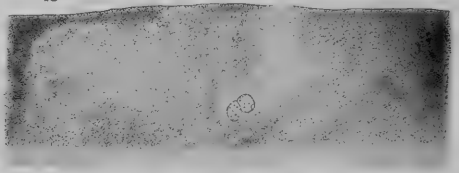

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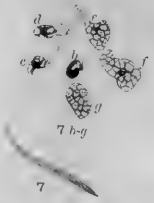

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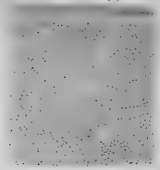

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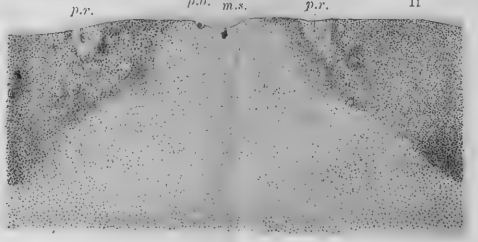

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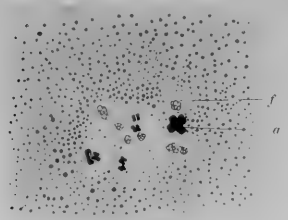

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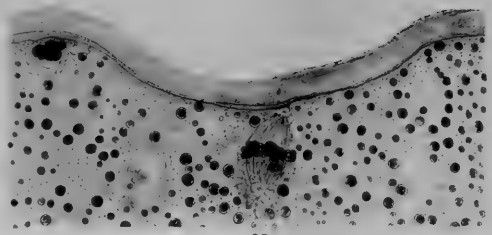

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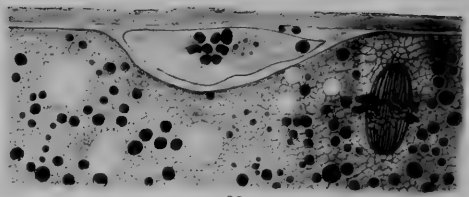

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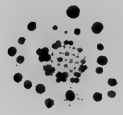

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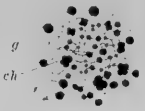

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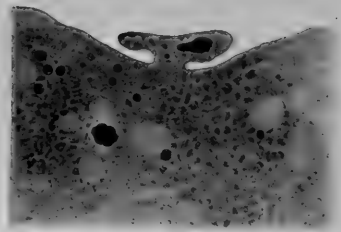

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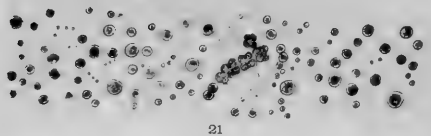

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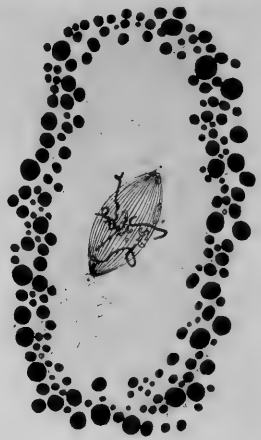

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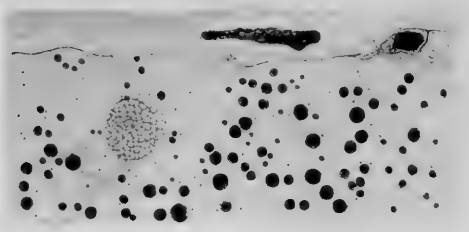

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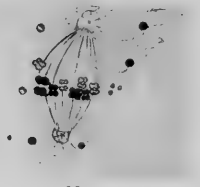

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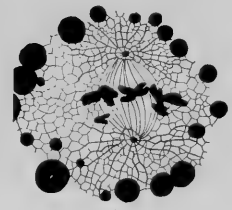

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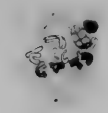

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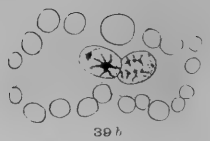

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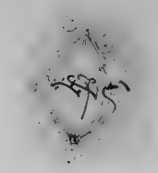

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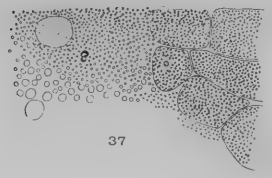

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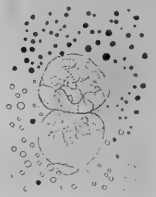

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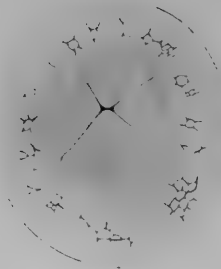

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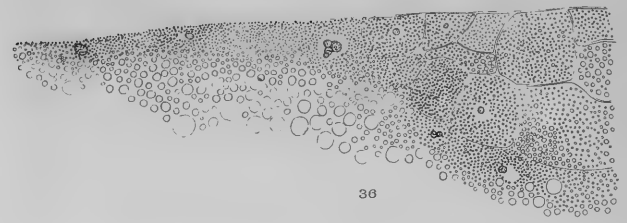

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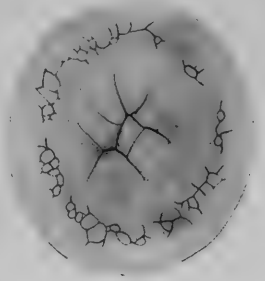

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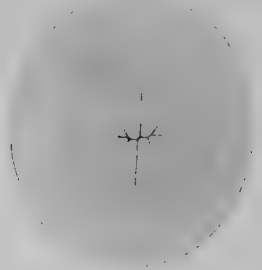

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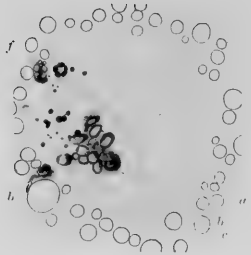

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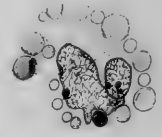

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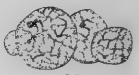

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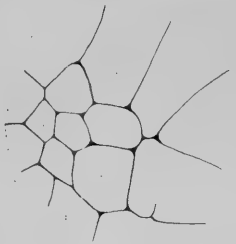

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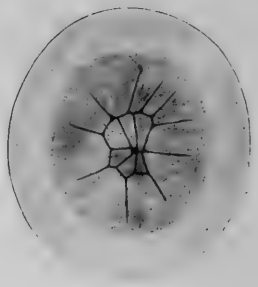

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DUPLICATE TWINS AND DOUBLE MONSTERS.

BY

HARRIS HAWTHORNE WILDER, PH. D.

From the Zoölogical Laboratory of Smith College.

WITH 2 PLATES AND 11 FIGURES IN THE TEXT.

Having recently called attention to the great similarity in the configuration of the epidermic ridges on the palms and soles of identical twins,[1] and seeing that the subject is one involving important biological problems, it has seemed to me of importance to collect as much evidence as possible on this head, and place it in convenient form, that it may serve as a basis for future speculation.

Furthermore, as identical or *duplicate* twins have not been generally defined save by the somewhat untrustworthy criterion of facial resemblance, and as their close relationship to certain of the types included under the head of *double monsters* has not been clearly emphasized, I have begun the paper with a discussion of the general subject. This portion of the paper, which presents a series of the most important data concerning twins and compound monsters, will serve as a necessary background for the facts presented in Part II, which constitutes the more original part of the investigation. Part III presents the deductions as far as they seem indicated, but is intended more as an aid in directing speculation in the future than as a set of dogmatic assertions which would be at present premature.

PART I.

MULTIPLE BIRTHS AND THEIR RELATIONSHIP TO COMPOSITE MONSTERS.

DUPLICATE AND FRATERNAL TWINS.—It is well known that there are, at least in the human species, two types of twins; the first include those cases where the sex may or may not be the same and where the general resemblance is about what may be expected in the case of any two children

[1] Cf. Palms and Soles, in Amer. Jour. Anat., Vol. I, p. 423, Nov., 1902.

of the same family; the second, those who are invariably of the same sex and who otherwise so closely resemble one another that it is difficult or impossible, especially during youth, for those not intimately associated with them to distinguish between them, the so-called "identical" or "homologous" twins.

Although these two types are both very common, the second rather more than the first, there seems to be in the popular mind no clear distinction between them. That there is a general impression that twins ought to look alike appears from the emphasis placed upon cases where they do not, but that this identity of facial expression does not extend to twins of opposite sex is a fact not commonly apprehended, and instances in literature are not rare in which a young woman in disguise is passed off as her twin brother, or the reverse. As a matter of fact all twins of opposite sex, as well as many cases in which the sex is the same, belong to the first, or non-identical type, while for the second type an identity of sex as well as of facial expression and other bodily peculiarities is a prerequisite.

Concerning the nature of this peculiarity, the most plausible and, in fact, the only hypothesis is that twins of the identical or homologous type are produced by the division of a single fertilized egg, while the other type results from the fertilization of two separate eggs, either from the same or different ovaries, and are thus two fundamentally distinct individuals, *i. e.,* a case of multiple birth such as normally takes place in most species of mammals. As expressed recently by Weismann, **02** (II, p. 54), "Wir haben nun allen Grund, die erste Art von Zwillingen (*i. e.,* fraternal) von zwei verschiedenen Eizellen abzuleiten, die letztere Art aber (*i. e.,* duplicate) von *einer einzigen,* welche erst *nach* der Befruchtung durch *eine* Samenzelle sich in zwei Eier getheilt hat."[2] Corresponding

[2] It seems impossible, with any degree of certainty, to place the credit for the first enunciation of the above hypothesis. Although often attributed to Francis Galton, Baudouin, 91 (p. 274), ascribes it to Camille Dareste, the noted teratologist, who in 1874 defended this theory before the Societé d'Anthropologie against the opposition of Paul Broca. Fisher, however, in 1866, antedating the statements of either of the above on the subject, advances the same hypothesis to account for the formation of double monsters, stating that they "are invariably the product of a single ovum, with a single vitellus and vitelline membrane, upon which a double cicatricula, or two primitive traces, are developed" (66, p. 208). As Fisher published in a magazine not readily accessible, at least at that time, to foreign investigation (Trans. Med. Soc. State of N. Y.), and as the similarity of separate and united duplicates might not have appealed to them, the formulations of both Galton and Dareste may well have been arrived at independently of Fisher's theory, and the same ideas may have occurred also to others working in the same field, since the hypothesis is of so obvious a nature.

to this hypothesis, which, in the light of our present knowledge, appears to be not far from the truth, we may designate these two types respectively as *Fraternal* and *Duplicate,* thus doing away with the misleading and inapplicable terms "identical" and "homologous" as applied to the one type, and furnishing a distinguishing term for the other, which seems thus far to have remained without a name.[3]

Intra-uterine Relations.—As the discussion of the origin of these two types of twins leads us to the consideration of the conditions which obtain during early embryonic life, we naturally turn to the observations furnished by obstetricians; but this source, although supplying numerous illuminating facts, is less valuable than it should be, owing to the fact that medical men share the popular confusion noted above in regard to twins and that, while they record trustworthy details concerning placentation and other relationships, *they fail to correlate with these the necessary data concerning sex and general resemblance,* the last item of which involves the following up of the case through several years of development, a line of work hard to accomplish during active professional life. The most noteworthy set of data covering these points are those tabulated by O. Schultze, 97, who gives in the form of a classification the various intra-uterine relationships which have been observed in twin births, with suggested correlations of the type of twin produced in each case. As this table is so essential to the present inquiry, I will transfer it in a somewhat abbreviated form, modifying its very accurate terminology to conform to that in more general use.[4]

Intra-uterine Relationships in Twin Gestations.

Case I.—Two separate blastodermic vesicles with two deciduæ reflexæ and two placentæ. This case is probably one in which there are two separate eggs, either from the same or from opposite oviducts, and implanted

[3] Strictly speaking, the word "fraternal" applies only to twins of the male sex, since in Latin, as well as in English, there is no word which, like the German "Geschwister," applies to sisters and brothers alike. The present use of the word in question, however, corresponds to that of the English masculine pronoun "he" in similar cases, and thus seems entirely warrantable. Pearson's term "Sibling" is correct in meaning, but is so rarely used that I hesitate to employ it.

[4] Schultze confines the term "Keimblase" (blastodermic vesicle) to the blastula stage of the embryo, employing for the later stages, to which the same term has been generally applied hitherto, the term "Fruchtblase." The parts surrounding this and supplied by the uterine mucous membrane are termed collectively "Fruchtkapsel," the free portion of which is the decidua capsularis (decidua reflexa autt.).

at some little distance from one another. In one case investigated by v. Kölliker, the two deciduæ were distinct but partially adherent over the surfaces in mutual contact, and in another the contact surfaces had fused into a single wall into which, from the two opposite sides, the chorionic villi of the two embryos had grown. In addition to this, one of the placentæ was of the type known as a placenta marginata, caused by a fold of the decidua. [This case is evidently a normal multiple birth, a condition hard to accomplish in a uterus of the shape found in human beings, and often attended by such phenomena as adhesions, fusions and foldings, all indicative of crowding and of nothing else.]

Case II.—Two separate blastodermic vesicles enclosed in a single decidua. Placentæ fused with one another but with two separate sets of umbilical vessels. Two chorions, fused at the point of contact. This case is more frequent than (I) but apparently results from the same general cause, *i. e.,* two separate eggs, which are, however, implanted nearer together. This would seem more likely to happen if both eggs came from the same side. [The conditions are seen to be similar to those of (I), the greater degree of fusion being well accounted for by the greater approximation of the two eggs to one another.]

Case III.—Two amnions and two umbilical cords but with a single placenta, in the middle of which the two cords meet and upon which the umbilical vessels closely anastomose. These are enclosed in a single chorion and covered by a single decidua reflexa. This case is said by Hyrtl to be more frequent than (I) and (II) but is not as frequent according to Späth. The twins are always of the same sex. Schultze says that the explanation of this singular condition is "zweifelhaft," and gives the following possible explanations: (1) At first two chorions, as in (II), the contact wall between which becomes absorbed later; (2) may have come from a single egg with double yolk, or (3) from an ovarial egg with two nuclei (cf. v. Franqué, 98, Stoeckel, 99, H. Rabl, 99, and v. Schuhmacher u. Schwarz, 00). It is conceivable that from such an egg as this last two blastodermic vesicles and two chorions could develop within one zona pellucida, at a later stage of which the two chorions could fuse. V. Kölliker considers it more probable, however, that in such a case the egg would develop two embryonic areas upon a single blastodermic vesicle and that a single chorion would then be the natural result. Each embryonic area would develop its own amnion. In this case the two allantoides would necessarily fuse, being included in a single chorion, and there would come to be between the two embryos a single (common) yolk-sac with two yolk-stalks. V. Kölliker has observed such cases in hen's eggs (but without the fusion of the allantoides). M. Braun

has seen it in lizards and Panum describes separate embryonal areas upon one yolk (hen's egg). See also Kaestner's figure of a double egg of *Pristiurus,* 98. [This case seems to put us on the right track regarding the origin of duplicate twins, especially since it is stated that the twins are always of the same sex, and although observations of later physical identity are wanting, it seems safe to assume it. It would seem hardly probable, however, that duplicate twins would arise from an ovarial egg with two nuclei, since in such a case the fertilization could be effected only by means of two spermatozoa, thus introducing different paternal characters; but if we reject all of Schultze's alternatives and substitute the possibility suggested above, that of the complete separation of the two blastomeres resulting from the first cleavage of a fertilized egg, the two components would still remain within one zona pellucida and would later become enclosed within a single chorion, which would develop a single placenta to which each allantois would later become attached. Each blastomere would undoubtedly form at first an independent blastodermic vesicle but the close association of the two would readily tend toward a fusion of the contact surfaces, thus forming a single vesicle upon the surface of which are two embryonal areas. If far enough apart from one another, each would develop its own amnion, but if near together a common amnion would result, thus producing the condition given in Case IV. This whole matter of the actual condition of the development of two closely associated embryos is very obscure, as there are but scattered and insufficient data bearing upon the case. It will receive a more extended consideration later on, under the headings "Origin of composite monsters" and "Other recent theories concerning the genesis of composite monsters."]

Case IV.—Similar to (III), but with both embryos enclosed in a single amnion. This is a very rare case, explicable only by postulating a single blastodermic vesicle upon which the two embryonal areas are nearly or entirely in contact with one another, a case which has been described by several authors as occurring in the hen's egg. In such a case there would be an almost irresistible tendency towards the fusion of the two embryos along the line of mutual contact, thus producing some form of composite monster. (Schultze says: "Doppelmissbildungen," but I use the word *double* in a more restricted sense as explained below.)

[As Case II is seen to be a variation of Case I with the two embryos nearer together, so Case IV is seen to be a similar variation of Case III, with a similar result, *i. e.,* the more complete fusion of parts, although here, owing to the direct connection of the two embryos the fusion is liable to extend also to these and produce abnormal results. There are

thus primarily, not four but two cases, corresponding to the two types of twins, Fraternal and Duplicate. The close connection of IV and III suggests what may have already occurred to the reader, that many cases of compound monsters come under the same category as separate duplicates. This is quite probable, but such forms, arising from a secondary fusion, would be asymmetrical and more or less unequal, and would come under the class of autosite and parasite rather than that of symmetrical, or genuine double, monsters.]

Definitions of Duplicate and Fraternal Twins.—These considerations, together with the distinctions made at the beginning of the article, will enable us to formulate distinctive definitions of the two forms of twins, as follows:

I. *Fraternal Twins.*—Either of the same or opposite sex and bearing no closer physical resemblance than is usual in children of the same family. These probably originate as two separate eggs, and any intimacy of association during intra-uterine life (which is never as close as in duplicates) may be attributed to the crowding within narrow limits to which they are necessarily subjected and for which no adequate provision is made such as occurs in mammals in which multiple births are the rule and not the exception.

II. *Duplicate Twins.*—Invariably of the same sex and exact or approximately exact physical equivalents of one another, especially in youth, before the modifying influences of environment and habit have had much opportunity to affect them. During intra-uterine life these are more intimately associated than are other twins, and in rare cases this association is of so close a character as to result in the production of compound monsters. All such cases, whether separate or united, may be referred to one and the same cause, that of some division in the fertilized egg, presumably that of the first cleavage nucleus, in such a fashion as to result in the formation and development of two embryonal areas upon a single blastodermic vesicle.

Triplets and Other Multiple Births.—The subject of twins and their intra-uterine relations is not complete without reference to the similar phenomena presented by triplets and the rarer cases of higher numbers at a single birth. According to the statistics of Veit the review of thirteen million birth records in Prussia shows that cases of twins occur once in every 88 births, triplets once in 7910, and quadruplets once in 371,126, and Norris, **96**, states that twin births occur in New York and Philadelphia in the proportion of 1 to 120, while in Bohemia the proportion is 1 to 60. Mirabeau, **94**, states that triplets are most common in multiparous women between thirty and thirty-four years of age,

where they occur once in 6500 births. Above quadruplets authentic cases are, as might be expected, very rare, but the Index Catalogue of the Surgeon-General's Library at Washington reports (according to Gould and Pyle, 97) 19 cases of quintuplets and two cases of sextuplets. A case of seven at a birth is recorded, according to Barfurth, upon a memorial tablet of the year 1600, found at Hameln an der Weser, and the Boston Medical and Surgical Journal of Sept. 26, 1872 (Gould and Pyle) gives numerous authentic details of a case in which eight children, all alive and healthy, but rather small, were produced at a single birth. This number may serve as a limit for authentic cases, but numerous mediæval authorities are considerably more liberal in the matter.

Our immediate interest here centers about the details of intra-uterine relationships, and of sex and general resemblance; and, as might be expected, details are very meagre and are often lacking in particulars quite essential to the present argument, although data enough have been discussed here to render it probable that in multiple births over two in number, the same two classes exist as in the case of twins, and that the individuals of a set may be all duplicates, or all fraternal, or, what seems to be more common, both sorts may exist in the same set. When larger numbers than three are involved, it seems possible to divide the individuals into two or more groups in accordance with this distinction; thus in quintuplets two may be duplicate twins, while the other three may form a set of duplicate triplets, if the expression be allowed, or there may be two sets of duplicates and a fraternal member, and so on.

As in determining the type of twins, the three sets of data which are of use here are (1) the intra-uterine relationships, (2) the sex and (3) the general physical appearance, and it seems thus far impossible to obtain all three sets of data in any one instance. The conclusions are, therefore, in the line of inference, but as such, particularly with the study of twins to guide us, they seem fairly safe, and may be utilized as prophesies or *a priori* deductions with which the data obtainable in the future may be compared.

The obstetrical phenomena observed in these cases are not numerous, but taken in connection with the similar study of twins, are extremely suggestive. Schultze says that in instances of triplets his Case III (see above), with a single chorion, has been noted, and also Cases I and II, with separate chorions. [The first instance is evidently a case in which all the individuals are of the duplicate type and the others are undoubtedly fraternal.] In another case one blastodermic vesicle was independent and distinct from the others, while the other two were related as in Case III [evidently two duplicates and one fraternal]. Sperling reports a

case like the second one of Schultze in which each individual had its own chorion, amnion and placenta, and in which both sexes were represented [fraternal type]. In a case of quintuplets (Schultze) the five individuals were divided into two groups, one of three and the other of two, each group with one placenta and a single amnion. [Probably a set of twins and a set of triplets, each of the duplicate type, and born at the same time, *i. e.,* the groups were fraternally related. The fact of the enclosure of the members of each group within a single amnion interprets this as two simultaneous instances of Case IV, and suggests the danger of the fusion of the individuals of each group into a composite monster, owing to the necessary proximity of the embryonal areas.]

Concerning the possibility of sex in multiple births, it is evident mathematically that triplets must be either of the same sex or else two must be of one and one of the other. None of these cases postulate much concerning the intra-uterine conditions or the type of individual, except that where both sexes are represented, they cannot all be of the duplicate type. In such a case the two that are of the same sex may be duplicates or not. In quadruplets, of which reference to 72 cases is found in the Index Catalogue of the Surgeon-General's Library at Washington (Gould and Pyle) the case becomes still more complicated and practically nothing can be postulated from sex data alone save a certain number of probabilities. In one case, for instance, two girls possessed a single placenta between them while the two others, apparently girls also, had each her own placenta. Here little can be told owing to the insufficiency of data, but it may be surmised that the first two were duplicate and the last two fraternal. Another case reported in which there were two boys and two girls, all united to one placenta, is a little difficult to classify, but the union of placentæ may have been due in part to the close approximation, and it is possible to consider the case one of two sets of duplicates, related set to set as in Case II.

As regards quintuplets, I have seen reports of the following combinations of sexes, although the other data were insufficient to draw any conclusions whatever: ♀♀♀♀♀, ♂♂♂♂♀, ♂♂♂♀♀, ♀♀♀♂♂.

In each of two cases of sextuplets there were four boys and two girls and in at least one of these (Vasalli, **88**) there was a common placenta for all. In the only authentic case of octuplets which I have been able to find (Boston Med. and Surg. Journal, Sept. 26, 1872) there were five boys and three girls.

Concerning the third set of data, that of physical identity, although of extreme importance in the present argument, no mention is made in any of the cases above quoted, evidently because they were the reports of

obstetricians who had no opportunity of following the cases into later years. Another cause of this lack of evidence is the great liability of the death of at least one of the set before they have matured sufficiently to show individual characters. We are thus forced to depend upon such data as can be obtained concerning older children and adults, in which cases the intra-uterine conditions are no longer obtainable, and it seems well-nigh impossible with such observations as have been taken up to the present time to obtain the three sets of coördinate data from any one case. This has resulted in part from the difficulties in the way of obtaining data requiring observations several years apart, but in great measure also from the lack of theories to show what data are needed, and thus each observer has obtained what seemed of interest to him. Although it is very evident that a busy practitioner during the rounds of his daily and often nightly visits has but little time for detailed observations beyond those called for by the actual needs of the cases, yet learning is advanced by just such data as those which he has the opportunity to collect, and it is by the compilation of facts like these that most important generalizations may be ultimately obtained. Any facts obtained and communicated to the writer or to any one else at work upon the theoretical side of the subject will further the advance of general knowledge in this field.

So far as I have been able to learn there is, as in the case of twins, a general belief that triplets and quadruplets ought to look very much alike, but the data obtained from the placental conditions certainly suggest that cases of fraternal components may also occur, either with or without the combination of duplicate components in the same set. One sees occasionally photographs of duplicate twins or even quadruplets employed for the purpose of advertising some infant's food or similar goods, but, although the probabilities are that they are authentic, there are numerous possibilities of deception known to modern photography, even to the repetition of a single person upon one and the same plate, thus rendering data from these sources a little too unreliable for use in this place. I have obtained, however, a genuine case of triplets, the components of which are all duplicates of one another. A photograph was taken of these at the age of eighteen and exhibits a remarkable degree of resemblance. In early life the physical identity of these triplets must have been complete, as the following extract will show, taken from a letter concerning them written by a lady who, when a young girl, knew the triplets as children. "I have seen twins that looked very much alike, but I could see a difference when they were together. I could not see any difference in these triplets when they stood in a row before me, and I never saw any one else who could, except their mother. She said she

could, but I doubted it; they used to fool her often. When they were babies she kept different colored beads around their necks to tell them by. They always weighed on the same notch until they were seven years old, then one gained half a pound more than the others." [5]

Duplicates among Lower Animals.—It is altogether likely that the phenomena of duplicate twins and other similar combinations are not confined to man but that they are more or less common among the lower animals. In mammals that produce several young at a time, it is probable that the components are mainly fraternal, but it is also likely that there may be occasionally one or even more sets of duplicate components in a given litter among their normal and contemporaneous brothers and sisters.

Observations upon this point are best made by a study of the intrauterine relationships, although in piebald domestic animals there is often sufficient individual differentiation to render possible observations along the line of personal resemblance, characters in color and marking taking the place of those in facial expression. Regarding lower vertebrates, especially birds, a large number of instances have been recorded, some of which are of interest in this connection. Thus, v. Kölliker describes a hen's egg containing two embryos, each with its own amnion and allantois, but sharing betwen them a single yolk to which each was attached by its own independent yolk-stalk, and M. Braun has noted a similar condition in the lizard. These cases are cited by Schultze and placed by him under his Case III, given above. The mature results of these would certainly have been duplicate twins, either separate or united in the umbilical region. For invertebrates the very numerous experiments of Wilson, Morgan and many others, performed upon the alecithal eggs of numerous marine forms, and in which separate individuals are formed by shaking apart the early blastoderms, suggest that the same result may occasionally take place spontaneously. The individuals thus artificially produced are undoubtedly genuine duplicates, and the process seems in every way comparable with the phenomena postulated above as occurring in the vertebrates, making allowance for the complications introduced in the latter case by the presence of yolk-sac and other extra-embryonal organs.

[5] When they were little girls one of them confided one day to a friend that she had been bathed three times that morning, while the others confessed that they had not been bathed at all, an incident that emphasizes their complete bodily identity at that period.

Relation of Duplicate Twins to Double Monsters.—The fact that in these experiments with invertebrates an incomplete separation of the components will produce various types of double monsters suggests that certain instances of these latter among vertebrates such as have been especially recorded in the case of man and other mammals, may be due to a similar origin. The opposite principle, also, that of the fusion of what were originally either separate eggs or separate blastoderms, seems also to obtain in some instances, as would be very apt to be the case where two blastoderms are enclosed by a single amnion (Schultze's Case IV), "ein Fall . . . der den nachsten Uebergang zu den Doppelmissbildungen darstellt," but in this case the two resulting compounds would not be symmetricaly joined nor of equal development.

Aside from these and similar speculations, nothing is definitely known concerning the real origin of either equal or unequal composite monsters, although these phenomena have been a favorite subject for speculation in all ages, and have given rise to numberless theories. Of these the most plausible seem to me those based upon the experiments with the eggs of invertebrates and upon the intra-uterine relations just considered, but, although we have these phenomena on the one hand to serve as causes, and the various types of composite monsters as results, the connection between the two is still mainly a matter of conjecture, and we are far from being able to explain definitely the relations between the various causes and the equally varied results. It is thus merely as a working hypothesis, upon which to base the facts presented later on in the paper, that I shall attempt here a discussion of the relationships of twins of both sorts to composite monsters, the relationships of the various types of these monsters to one another, and the probable causes which lead to the production of each type.

To present the material for this discussion before the reader, the following list of recorded instances has been compiled, whenever possible from the descriptions of actual observers; much also has been obtained from the various compilations referred to at the beginning of the bibliography. To all these sources I wish to acknowledge my indebtedness.

The classification adopted for this list is purely a geometrical one, and differs but little from that of other authors, the main attempt being to arrange the material in a convenient form for later reference. Later on, in discussing the origin of these monsters, they will be arranged in accordance with their probable physiological relations, in an attempt to show the causes of these phenomena. These relations are expressed also in the general diagrams (Plate A.).

Classified List of Composite Monsters.

I. DOUBLE MONSTERS IN WHICH THE COMPONENTS, OR COMPONENT PARTS, ARE EQUAL TO AND THE SYMMETRICAL EQUIVALENTS OF ONE ANOTHER. DIPLOPAGI.

These forms show, wholly or in part, two duplicate components, which are the equivalents of one another in size and development, *i. e.*, homo-dynamous; and are symmetrically placed as equivalent halves of the composite body of which they form a part. Occasionally, through lack of space, limbs and other parts which lie upon the inner aspect of the components, near the line of fusion, become more or less suppressed in their development, which may lead secondarily to a lack of complete symmetry in this region. (Exs. Osborne's calf, Blanche Dumas; v. infra.)

1. Each of the Two Components Complete or Nearly So.

Cases included under this division, of which the Siamese twins form a noted example, are plainly duplicate twins, in every respect like normal ones, but with a slight bond of connection uniting them, the position of which may vary but which is so placed as always to arrange the components symmetrically with reference to it, *i. e.*, the bond is confluent with each twin at the same spot anatomically, but, when other than median, the connection is with the right side of one and the left side of the other. The components possess the same degree of physical identity as is seen in normal duplicates.

a. Connection in or near the sternal region, usually median, so that the components stand face to face, sometimes more lateral. Thoracopagi. Type: Siamese twins, "Chang-Eng"; male; born in Siam, 1811. Examined at Harvard University in 1829, by Warren. Exhibited throughout the United States and several European countries (exhibition forbidden in France on account of the possible influence upon pregnant women!). Finally settled in North Carolina as farmers, under the name of Bunker. Both married. Chang had six children and Eng five, all normal. They died, almost simultaneously, in 1874.

Other cases:

1. Hindoo sisters, described by Dr. Andrew Berry, 1821.
2. Newport (Ky.) sisters, Austin Medical Gazette, 1832.
3. Orissa (India) sisters, "Radica-Doodica," b. 1887.
4. Arasoor (India) sisters (mentioned by Gould and Pyle, p. 171).
5. Two sisters spoken of by Swingler; operated upon and both lived.
6. Swiss sisters, "Marie-Adele," b. 1881. Separated at age of five months, but both died.

b. Connection by the heads, in various regions, usually median. Craniopagi. Type: Geoffrey St. Hilaire's case. Two sisters, b. 1495 and lived to the age of ten; joined at foreheads, otherwise entirely normal; when one died an unsuccessful attempt was made to separate the other.

Other cases:

1. Daubenton's case. Union was at occiput; farther details fail.
2. St. Petersburg case. 1885. "So united that the nose of one, if prolonged, would strike the ear of the other." This description is hardly clear, and at first thought appears to violate the law of

symmetry observed in other cases; probably a juncture at the side of the forehead.

3. A case is reported by Villeneuve, 31, in which the two components are united by the tops of the heads, extending in opposite directions, like *ischiopagi*, only joined at the anterior instead of at the posterior ends. They are placed in such a manner that they face in opposite directions. This is figured by Förster in his Taf. III, Fig. 16.

   (An exact counterpart of this, occurring in a hen's egg, was found in my laboratory in 1895, but unfortunately was not preserved. The chicks were of about the third day and were united along the top of the curve formed by the brain at that age, the heads turning in opposite directions.)

4. With some doubt there may be referred here the case cited by Home, 1790. In this a single child possessed an inverted head joined to its own, vertex to vertex, the face of the extra head being directed towards the right side of the child. As the supernumerary head was of full size and perfect, and furthermore as there was cicatricial tissue at the neck, it may well be supposed that this head once had a body equal to the other, and that it had suffered an early amputation at the neck. (See also below, under II, 1, *c*.)

c. Connection at sacrum, the components being placed back to back. Pygopagi. Type: Bohemian twins, Rosalie-Josepha Blazek. These sisters were born at Skreychor, Bohemia, January 20, 1878. They were examined at the age of six months by Dr. August Breisky of the Medical Faculty at Prague. At thirteen they came to Paris and were exhibited to the public at the Theatre de la Gaité, and were carefully examined at that time (Baudouin, 91). They seemed to have been joined originally back to back, by a flexible connection in the sacro-coccygeal region, but their habitual attitude is such that they face in the same direction, each one diverging about 45° from the direct forward position.[6] The planes of the two chests thus form nearly a right angle. The four iliac bones bound a double pelvis, and the four nates include a quadrilateral space, within which are the external organs of a single individual, but with double internal connections, one for each component. The arrangement of these parts is not clear in Baudouin's description.

Other cases:

1. Negro sisters, Millie-Christine, b. North Carolina, 1851. Exhibited in United States and in France (Paris, 1873).
2. Tynberg's case. New York, two sisters, b. 1895. Reported by Jacobi in Archives of Pediatrics, October, 1895.

Aside from these more recent cases, there are numerous well authenticated ones of earlier times. Of these (3) the Hungarian twins, Helena-Judith, are, perhaps, the best known. They were born in 1701 and lived to the age of 22. (4) A pair of Italian female twins of this type were born in 1700, and

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[6] Thus Baudouin, 91, describing Rosa-Josepha Blazek: "En les voyant assises à côté l'une de l'autre sur le même fauteuil, on soupçonne à peine leur union, quand elles sont habillées. Mais à peine l'une d'elles fait elle un léger mouvement, l'autre suit immediatement et se déplace en même temps."—*loc. cit.*, p. 273-274.

at the age of five months succumbed to an unsuccessful attempt to separate them. The famous Biddenden maids (5), Mary-Eliza Chulkhurst, appear to have belonged to this type. They were born at Biddenden, Kent, England, in the year 1100, and lived thirty-four years. In accordance with the conditions of a legacy left them, there is still in that place an annual distribution of food combined with several curious customs (cf. Teratologia, 1894-95).[7]

[The region of attachment of pygopagi often involves the outlets of the pelvic organs, producing various relationships in the different cases. Thus in the Bohemian twins there is a single anus, a single urethra, but two vaginæ, two recti and two bladders; in Tynberg's case, there are two vaginæ and two urethræ, but a single anus with a double rectum, divided by a perineum. In others there seems to have been two complete sets of these parts.

d. Connection at ischia, so that the axes of the two bodies extend in a straight line, but in opposite directions (Ischiopagi). Anus and genitals laterally placed between the legs of the same side.—These cases are said to be quite numerous, but seldom if ever live beyond infancy.

Type: As there seems to be no especially celebrated case to serve as a type, I will present a fac-simile of an old engraving (Fig. 1) evidently drawn from nature, and portraying a "Missgeburt" that occurred in Hanau, near Frankfort, in March, 1643. This type is so accurately portrayed in the engraving as to reflect great credit upon the artist and upon the genuine scientific observations of the time. It is of interest to compare this with the photographs of the Jones twins, given by Gould and Pyle, p. 183.

Other cases:

1. Oxford (England) sisters, b. 1552, lived but a short time.
2. Paré's twins, b. 1570, and baptized Louis and Louise. This seems to imply that they were of opposite sexes, but this is intrinsically improbable. It is more likely either that the record is not wholly trustworthy or that the twins were both boys, since girl's names were frequently bestowed upon boys during that epoch.

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[7] The Samoan legend of the "two swimming sisters of the sea" gives a most detailed and perfectly accurate description of female pygopagous twins, leaving it beyond question that the tale is in part a reminiscence of an actual case probably antedating any other record.

The fact of the union between these two sisters "is so clearly brought out in all the legends as to lead one to the belief that there must be at least this historic basis, that in Samoan antiquity there must have been a pair of twins united more or less extensively by connecting ligaments. Not only is the fact of the junction clearly dwelt upon, but the manner of the attachment is no less distinctly stated. This was by a ligament connecting in each member of the couple a point on the spine high up between the shoulder blades. The sisters were thus brought back to back; when one walked forward the other had to step backward; when one bent over to pick up anything on the ground the other was lifted in the air and borne on her sister's back. In every account of the twins explicit mention is made of these inconveniences and without the omission or alteration of a single material particular." [Quoted from Llewella Pierce Churchill, in Forest and Stream, August 24, 1901.]

3. Irish sisters, b. County Roscommon, Ireland, 1827, baptized Mary and Catherine. One was dark haired and the other blonde. (!)
4. French sisters, b. 1838, baptized Marie-Louise and Hortense-Honorine; exhibited at Paris, but soon died.

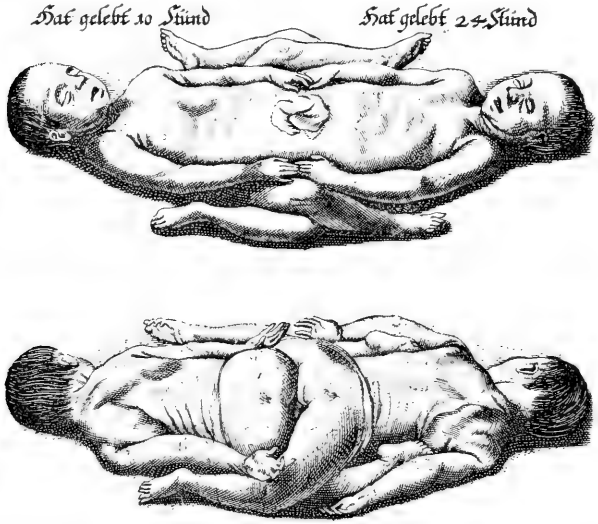

FIG. 1. Case of ischiopagous duplicates born at Hanau, near Frankfort, Germany, March 11, 1643. Fac-simile of a contemporary engraving in the author's possession.

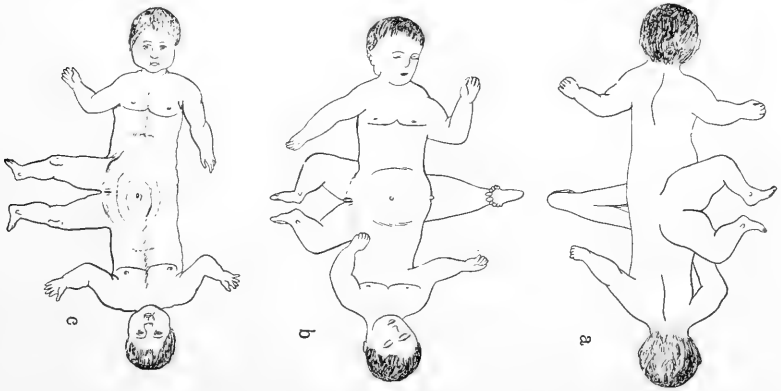

FIG. 2, *a* and *b*. Case of imperfect ischiopagous duplicates, showing upon one side a double bilateral limb, composed of halves belonging to each component. Born in Cadiz, Spain, May 30, 1818.

c. *Monstrum Anglicum*, born in Salisbury, England, October 26, 1664 [after Licetus].

5. Ceylonese brothers, b. 1841; exhibited at Colombo, Ceylon, but lived but a short time. Anus and genitals single.
6. Millville (Tenn.) sisters, b. about 1867; exhibited in New York.

7. Ohio sisters, Minna and Minnie Finley; described by Goodell in 1870.
8. Jones twins, b. in Tipton Co., Indiana, June, 1889; sex not given; exhibited for some time and died at Buffalo, N. Y., February, 1891.

Aside from typical cases like the above, in which both components are complete, with well developed legs, there are numerous instances in which the legs of one or both sides show a greater or less reduction, with a corresponding modification of the genitals and anus. Cases like the one figured here (Fig. 2 *a* and *b*) with one pair of legs represented by a doubly bilateral stump are of great interest, as coming under the same category as the median arms and legs of such cases as those of subdivisions 2 (*a*) and 2 (*c*) below.

The case figured in Fig. 2 (*c*) is logically of great importance, as it furnishes a connecting link between ischiopagi and such monsters as the Tocci brothers (2 (*d*) below). It may be considered either as (1) a case of imperfect ischiopagi like that of Fig. 2 with the median leg suppressed, or equally well as (2) monsters like the Tocci brothers with a greater divergence between the two bodies. The monster in question is the famous "*Monstrum Anglicum*," born to Mrs. John Waterman, Salisbury, England, October 26, 1664, and although the event is now so remote it rests upon unquestionable authority. At the same birth there was produced also a normal female child.

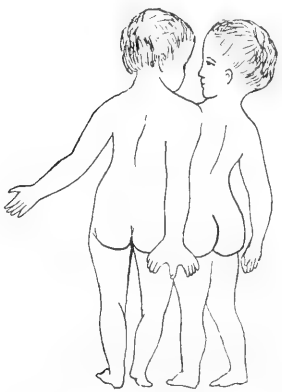

Fig. 3. Regnault's ectopage [after L. Blanc].

c. Connection along the side of the body, so that the components are definitely right and left. Inner arms represented by a bilateral median limb. (*Ectopagi.*)

Type: The only case which I am able to find is that of Regnault, figured both by Förster, *l. c.*, Taf. IV, Fig. 4, and by L. Blanc, 93, Fig. 111. This figure I have reproduced here (Fig. 3), as it serves as an interesting transition between this subdivision and the next.

Förster's description is as follows: "Weiblich; ein mittlerer Arm mit doppelter Hand. Brust und Bauch seitlich verschmolzen."

Were one to imagine the two inner legs represented by a bilateral median member, as in the case of the median arms, the monster would be the exact counterpart of Fenn's fetus or of the Würzburg case No. 75 (2 *c* below).

2. The Two Components Equal to One Another, but Each One Less than an Entire Individual.

These cases form a graded series, illustrating almost every transition from the cases cited under (1) to those of single and otherwise normal individuals, with a duplication of a restricted median area, like that of the genitalia. The doubling often affects one end of the body alone, thus producing a monster single below and double above, or double below and single above.

It is to be noticed that in cases in which the doubling involves the head there are two distinct personalities, one for each head, and that in each half of the undivided or median portion the sensation is referred to the head of the corresponding side, although there is usually a narrow zone of common

sensation along the median line. When the head is single there is but a single personality.

a. Components separate above and united in the pelvic region, but with a single perfect leg each, the outer in each case, while the two inner legs are represented by a double median appendage which is bilateral, and may be well developed or rudimentary.

These monsters and those included under (*b*) and (*c*), although existing mainly in the form of fetuses in museums, form the necessary connecting links between the last group and the type represented by the Tocci brothers. In these the inner arms or legs (or in rare cases both) are represented by double members which consist so plainly of two symmetrically placed components united together that one is strongly tempted to consider it a fusion of two originally independent limb components. Instead of this, in accordance with the theory supported by this paper, such double limbs must be looked upon rather as parts, the anlagen of which were incompletely separated, and were thus not allowed to develop independently of one another.

This and other similar points will be taken up more fully later on, when a résumé is made of all double forms with attempts at their explanation.

Type: Fisher's case 43 (quoted from Walter, Observationes anatomicas, Berlin, 1775). This monster was one of twins born to Anna Maria Woblack, near Berlin, November 17, 1773, all of the male sex. One of these was a normal, healthy child, the other the double monster in question. "The placenta was a single mass, to the opposite sides of which two cords were attached, one for the single, the other for the double fetus; the cord of the latter was three yards long. The normal and abnormal fetuses were both enveloped in a single and common chorion."

As for the double monster, the type of this subdivision, Fisher states that "the vertebral columns are complete in both (components). Between the two sacra there was found a shovel-shaped osseous mass, consisting of rudimentary fused iliac bones, from the lower part of which hung a median lower extremity, which was attached by ligaments. This compound posterior pelvic extremity contained a femur; a tibia, which was thick and bent at an angle; the usual tarsal bones; seven metatarsals, six of which formed a row; the seventh and largest supported two toes which were webbed."

Other cases:

1. Marie-Rosa Drouin. New-born female infant from Montreal, described by MacCallum in 1878.

The two complete upper components formed a right angle with the common trunk "which commenced at the lower part of the thorax of each." There was a rudimentary buttock between the two lateral ones, from which projected "a rudimentary limb with a very movable attachment. This limb, which measures five inches in length, and is provided with a joint, tapers to a fine point which is furnished with a distinct nail. It is very sensitive and contracts strongly when slightly irritated."

This case, with the rudimentary posterior limb, shows close similarity both to the type above (Fisher's case 43) and to those of (*d*) below, since, with the exception of this small median rudiment, the case is identical, save in sex, with that of the Tocci, including the median buttock, while on the other hand, it differs from the type of this subdivision merely in the lesser development of the median leg.

b. Like (a) but united from the shoulder downwards and with a median anterior limb, or limb-rudiment, and no median leg.

Type: Barkow's case, cited by Fisher, 66.

The two components were united from pelvis to shoulder, and were apparently single below the waist, with a single pair of legs. In place of two median arms, such as are seen in the cases under (*d*), a median bilateral member appears, attached to what is apparently a double shoulder girdle, formed of equal contributions from the two components. A sketch is given of the bones of this median limb, which shows an abnormally broad humerus, to which are articulated a median ulna (double) and two radii, laterally placed. The digits are ten in number, not palm and palm, as in Fenn's fetus above, but side by side, the ulnar sides at the median line (Fig. 4, *c* and *d*).

Other cases:

1. Gurlt's double calf (copied from Gurlt's Atlas by Fisher, Fig. 5).

This is so similar to the last that a detailed description is unnecessary. The duplication extended, however, farther down the back and there seemed to be two nearly complete vertebral columns and two tails.

2. Meckel's fetus. Carefully dissected, described and figured in a large folio, published 1815.

The inner arms are represented by a median rudiment which proceeds from a double shoulder girdle. The rudiment consists of two joints called *humerus* and *antebrachius* by the author.

3. Gruber's fetus (1859, "Thoracogastrodidymus I"), is of great interest here since it is like that of Meckel (2), except that the median arm rudiment is considerably smaller. There is but a step between this and the "Thoracogastrodidymus, Case II," of the same author, described below.

4. Zimmer's "*Dicephalus tribrachius*," figured by Förster, 61, in Taf. VI, Fig. 4, evidently belongs here. The fact that the left component suffers from other defects is plainly a mere coincidence, without significance in our present study.

c. Forms which, like (a) and (b), consist of two laterally united components, but with a median double limb, or limb-rudiment, from both anterior and posterior limb-regions.

Type: Fenn's fetus; described by J. Wyman; specimen in Harvard Medical School (Fisher, 66, pp. 276 ff.).

This is the best case of all to illustrate the real double composition of these median appendages, since neither the arm nor the leg is really rudimentary but both are of perfectly bilateral symmetry and show the two equal components which, had the anlagen separated a little more, might each have formed two separate and perfect members.

As this is so important a case, a rough tracing of Fisher's plate, with a detail of the median foot, is here presented (Fig. 4, *a* and *b*). The description of the median appendages is as follows: The arm is double and symmetrical, and the foot is "compound and provided with two groups of toes, of three each, one right and the other left, and a single large symmetrical toe arose from the middle of the back of the foot. This toe had a nail on each side" (Fisher).

Other cases:

1. Würzburg Pathol. Coll., No. 75. Three figures of the skeleton of this fetus are given by Förster, *l. c.*, Taf. VI, Figs. 5-7, and show a condition almost identical with that of Fenn's fetus.
2. Tulp's fetus, quoted by Licetus, in the appendix to his "De Monstris," ed. 1665. The description is accompanied with an excellent engraving and shows a specimen closely resembling Fenn's fetus, but with the double hand components complete and free as far as the wrists. Although Licetus is very unreliable and fantastic, this case is undoubtedly authentic, since it rests upon the authority of Nicolaus Tulp and is in strict accordance with other observed facts.

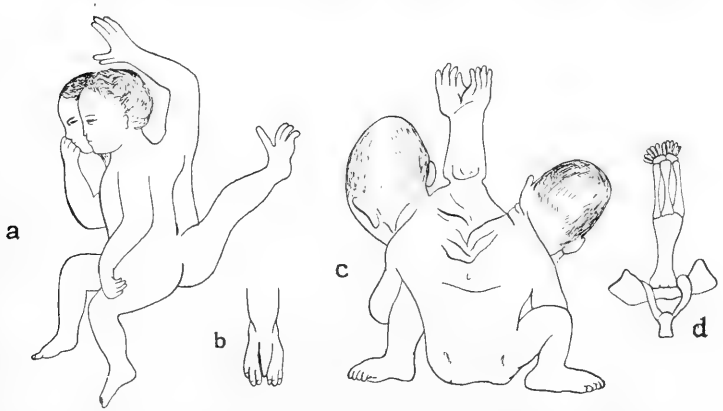

FIG. 4. Diplopagi with double median limbs.

(a) Fenn's fetus, described by J. Wyman. Specimen in Harvard Medical School Collection.
(b) Median foot of (a), dorsal view.
(c) Barkow's fetus.
(d) Skeleton of median arm of (c).

[All after Fisher, his figures 53, 54, 57 and 58.]

d. Components united at pelvis, above which they are distinct, each with head and pair of arms; the pelvis is often partly doubled, but with usually a single set of external median parts and with a single pair of legs, each one of which belongs to the upper component on its own side.

Type: Tocci brothers, Giovanni-Batista and Giacomo; born, province of Turin, Italy, 1887. The components were separate and normal down to the sixth rib, but possessed "a common abdomen, a single anus, two legs, two sacra, two vertebral columns, one penis, but three buttocks, the central one containing a rudimentary anus." A good photograph of these is reproduced in an article by H. L. Osborne, 02.

Other cases:

1. Normandy sisters, reported as having been seen in Normandy in 1062.
2. Bateman twins, male, b. in 1529.
3. Scottish brothers, b. about 1465; lived 28 years.
4. Swiss twins, male, b. 1598; lived at least 30 years. "So joined that at rest they looked upon one another." They married a single wife.

5. Ritta-Christina, b. Sardinia, 1829. They "joined in a common trunk at a point a little below the mammæ." They died at an early age.

e. Two separate and equal heads and necks upon a single trunk which is normal, or with some duplication at the shoulders, and with a single pair of arms. [In this especial care must be exercised to distinguish between cases in which the two heads are equal, *i. e.*, duplicates, and those in which one appears as an outgrowth or "parasite" of the other. Only the first of these belongs here, the other is not a case of true duplicates; in the one the components are homodynamous, in the other heterodynamous.]

Type: As there seems to be no recent case of a human monster of this sort, we may take as a type the two-headed turtle described by Barbour, 88. This is referred to also by Bateson, 94, who reprints Barbour's figure. This phenomenon seems to be especially common in snakes.

Other cases:

1. Double-headed girl of 1665; each head was baptized. It lived but a short time.
2. Milanese girl (of about the same date). Two heads, but the rest apparently single; after death she was found to have two stomachs.

(The numerous cases cited in which one head is described as ugly or deformed are doubtless to be referred to II, below.)

[A highly interesting case was dissected by Dr. Wenzel Gruber and figured by him in his memoir of 1859. Externally it would belong in this subdivision, but the skeleton displays two complete backbones, including coccyx; between which, in the sacral region there is a small spade-shaped piece representing the rudiment of the of the two internal ossa innominata.

The inner ribs are complete in number but strongly united, and there is a small rudiment of the inner shoulder girdle. Gruber figures this under the name "Thoracogastrodidymus, Case II"; his "Case I" of the same name possesses a visible median arm rudiment, and has been described above under (*b*).]

f. Like the last two, but with the two compound heads incomplete and united to one another.

Type: Moreau's case; exhibited in Spain. Possessed a fused head, with two noses and two mouths; each component had a perfect eye, the outer, while the two inner eyes were represented by a poorly developed median eye, with two pupils.

Other cases:

Buffon mentions a cat which was the exact counterpart of Moreau's case (see also Förster, Taf. I, Fig. 1-6).

A less marked class of defects, in which a single and otherwise normal individual possesses a double tongue, double uvula, or other doubling in the region of nose, mouth or throat, undoubtedly belongs here, and represents the process of doubling in its inception. These must be carefully distinguished, however, from cases of cleft palate, harelip, etc., which are due to a failure of perfectly normal parts to close in the median line, and which have no relation whatever to the present subject. (Compare the note below under diphallic terata (*i*)).

[Up to this point the cases cited are those in which the duplication becomes gradually reduced below while the two components remain distinct above;

the remaining cases cited under this subdivision will be those which show the opposite principle, that of duplication below with a single head and body. Here also the cases will be arranged in a decreasing series, ending as in (*f*) with a single body showing but a few traces of the two components.]

g. Two nearly complete components, joined front to front over more or less of the trunk region, but with a single neck and with the heads more or less completely fused into a single compound mass. The half-faces of the two components meet in the plane of union and form single faces of a varying degree of completeness, placed laterally with respect to the components. Janiceps.

These are the so-called Janus-headed monsters, two varying degrees of which are represented in Fig. 5. At first sight these monsters seem at variance with the general plan of diplopagi, since what appears to be the face

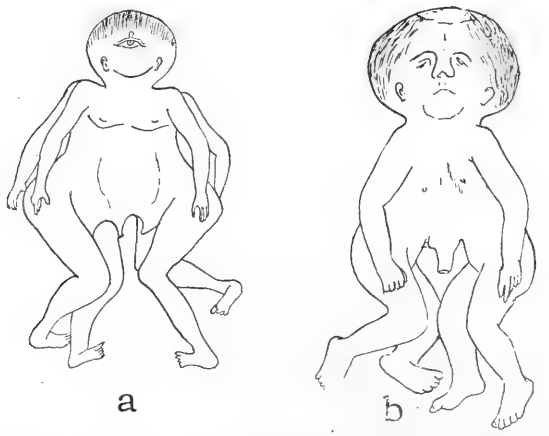

FIG. 5. Lateral view of two cephalothoracopagi (Janus monsters), showing different degrees of separation. The face which is towards the observer is duplicated in these monsters by another one on the opposite side, and thus from the dorsal aspect of either component there appear two profiles looking in opposite directions. Each lateral face is in reality composed of halves contributed by the two components.

components are placed laterally with respect to the trunks; but if the plane dividing these latter be conceived as passing through the faces also, it becomes evident that what seems a single face is made, in each case, of the right half of one component and the left half of the other, and that thus the two components are as in completely bilateral as in other cases. The case is identical with that of the laterally placed genitalia in ischiopagi.

h. One head and one trunk (and consequently a single individual), but with double pelvic organs and two pairs of legs (dipygus).

Type: Wells' case, Mrs. B., born May 12th, 1868, lives in Birmingham, Ala. Normal down to the third lumbar vertebra, where the duplication begins, resulting in a fused double pelvis, and two pairs of legs; of these latter the inner ones, although of normal shape, are somewhat reduced in size, either from lack of room for development or in part from disuse, since the outer

legs alone are used in walking; two sets of external genitals, normally placed with reference to the two pairs of legs; two bladders, two ani and two rectums. "The nates from below appear as those of two individuals with a distinct cleft between them. . . . . The functions of the duplicated organs are dual and entirely independent of each other, micturition and defecation occurring at different times on the two sides. . . . She was married shortly after her eighteenth birthday. . . . She is now in good health, is very intelligent, is perfectly able to attend to her household duties, and was twenty years old on the 12th of May, 1888." (Wells, 88, an illustrated account.) Shortly after her marriage, she became pregnant upon the left side, but an abortion was induced owing to the small size of the pelvic outlet. [The above is a most important case, stated by Wells to be the only known instance in man of such a complete doubling, although several cases have been observed in other animals. Gould and Pyle, however, refer to a case cited by Heschel in 1878, that of a girl of 17, with double parts below the second lumbar vertebra. There are certainly other known cases in which the duplicity is less complete and the inner legs more rudimentary, and of these the following will form a transition to the next type (*i*) of this subdivision.]

Other cases:

Blanche Dumas.

I am unfortunately unable to find any original description of this important case, and was at first utterly misled by the only illustration of her which I have seen, copied both by Wells and by Gould and Pyle. Here the pelvis appears incompletely double, and one is at first misled by the presence of but one leg between the two normal ones and that not a median but lateral one. It seems, however, that there is also a rudiment of a fourth leg, and we have only to admit the possibility of a check to its growth at an early age through lack of space, as was seen in the calf described by Osborne, 02, and the case becomes clear. The inner leg which appears in the illustrations seems to be the right one of the left component, while the rudiment is the left one of the right component. There are two distinct sets of genitals, presumably placed in the normal position relative to the four original legs as in Mrs. B. By the side of the rudimentary fourth leg there is a rudimentary mamma, an anomaly often associated with redundant lower limbs (cf. Louise L. and Bechlinger's case, given below). It is noteworthy that in both Mrs. B. and Blance Dumas, one of the outer legs, the left in both cases, exhibits talipes.

i. In most respects a normal single individual, but with a duplication of some or all of the median pelvic organs, often accompanied with a median leg rudiment composed of two united halves belonging to the two components.

Type: Jean Baptista dos Santos. A Portuguese (gypsy?), born at Paro about 1845, the subject of numerous papers (cf. London Lancet, August, 1865; also American reprint, January, 1866; Fisher, 66, etc.). The man was wholly normal save in the pelvic region, which showed an exact duplication of the external genitals, and a median third leg, depending from an extra median pelvic bone of unknown nature. The penes were normal and nearly equal in size, with a half scrotum on the outer side of each and a median scrotum evidently composed of the fused inner halves. The outer sacs held each a normal testis, and the median one originally contained two which ascended

into the abdomen at the age of ten. The median leg was double and apparently symmetrical at first, but it was dislocated in two places by the Portuguese chemist who officiated at his birth, that it might afterwards be doubled up and thus put out of the way. During later life he strapped it to his normal right leg and to his right side. This treatment well explains the slight lack of bilateral symmetry in the adult member. The foot bore ten toes, the two great toes in the center; the two outer toes of each component were webbed. In photographs of dos Santos as an infant the median member is quite symmetrical in spite of its injuries.

Other cases:

Bechlinger's case.

A female counterpart of dos Santos was reported from Para, Brazil, in 1888, by Bechlinger; the genitals were duplicated but otherwise normal and she possessed "a third leg attached to a continuation of the processus coccygeus of the sacrum, and in addition to two well-developed mammæ regularly situated, there were two rudimentary ones close together above the pubes."

Aside from the above there have been reported several cases of diphallic terata, all of which so far as known (20 cases) have been enumerated by Ballantyne and Skirving, 94-95. Of these one or two are doubtful, and some of the others do not come under the present head. Those that do belong here show "all the degrees of duplication . . . from a fissure of the glans penis to the presence of two distinct penes inserted at some distance from each other in the inguinal region." With these cases there is commonly associated the doubling of other topographically related parts; among these may be mentioned "more or less completely septate bladder, ... double anus, double urethra, increased breadth of the bony pelvis with defect of the symphysis pubis, and possibly duplication of the lower end of the spine." It must be noticed that in all of the cases here considered there is an actual doubling of certain of the median parts, although there may be at the same time a defect in the median line. This would sharply distinguish all such cases from those of episadias, hypospadias, etc., which are simply cases of arrested development, "Hemmungsmissbildungen," and are caused by the failure of two lateral anlagen to close in the median line. Compare the distinction between the cases of double tongue, double uvula, etc., mentioned above, and such defects as hare-lip and cleft palate.

II. UNEQUAL AND ASYMMETRICAL MONSTERS, ONE COMPONENT OF WHICH IS SMALLER THAN AND DEPENDENT UPON THE OTHER. AUTOSITE AND PARASITE.

These monsters consist of two components of very unequal development, the one (autosite) being normal or nearly so, and the other (parasite) quite incomplete and attached to the first as a dependent growth, usually adhering to some point upon the ventral side.

To my knowledge this form of monster has never been studied for the purpose of testing whether or not the two components were ever originally physical duplicates, and the relation between them is such, that is, the subordination of one to the other, that, even in cases in which the parasite is

furnished with a face, it is altogether probable that any original similarity of features would have been lost. In the records of cases accessible to me in which the parasite possesses sex, it seems always to be the same as that of the autosite, but that in itself is no proof that the components were originally duplicates, although, in accordance with the theories advanced here, it seems probable that the latter is always the case.

This class of monsters seems harder to arrange in groups than does the preceding and the following attempt is mainly for the purpose of putting the various cases in a convenient form for consideration.

1. Parasite Attached to the Outside of the Autosite.

a. Parasite possessed of a head, or head and arms, usually attached to the autosite at or near the epigastrium.

Type: Lazarus Johannes Baptista Colloredo, b. Genoa, 1617. Exhibited all over Europe. Autosite normal, bearing at the lower sternal region a parasite which consisted of a trunk, one thigh, two arms and a well formed head. The parasite gave signs of independent existence, but the eyes were closed and nothing was ingested by its mouth. The man was examined by Bartholinus. This case seems to be unique in the completeness of the parasite, but several other cases, in some of which the parasite consisted of a head alone, are on record.

Other cases (less complete than the type):

1. Dickinson's case, 1880. Child of five years, with a supernumerary head attached by a broad base to the lower lumbar and sacral region.
2. Havana case. Examined by Montare and Reyes. Girl of seven months with an imperfect accessory individual attached between the xiphoid cartilage and the umbilicus. The accessory head was imperfect, but had hair; the parasite had in part a separate sensation.

Moreau's case. Infant born in Switzerland in 1764. The parasite seems to have resembled that of Colloredo; it was amputated by a ligature.

b. Parasite consists of the legs and more or less of the lower part of the body, without a head; attachment to autosite as in 1.

Type: Laloo, Hindoo boy born about 1876; exhibited in dime museums all over the United States. The parasite was dressed in girl's clothes and was said to be female, but was in reality a male. The parasite possessed arms, but no head, and had no separate sensation.

Other cases:

1. A-Ke, a Chinaman, exhibited in London early in the nineteenth century, and now largely represented by wax models in continental museums. Case similar to last.
2. Louise L., "La dame aux quatres jambes," born in 1869. Parasite consisted of two atrophied legs on a rudimentary pelvis, attached ventrally to the normal pelvis; two rudimentary mammæ at insertion of legs. "The woman could localize sensation in the parasite except in the feet." She married and bore two normal daughters. [In some respects, *e. g.*, the rudimentary mammæ, this case is strikingly similar to Blanche Dumas and to Bechlinger's case (see above), except that here there is no doubling of parts in the autosite.]

3. Gustav Evrard. Case described by Guerin. The parasite consisted of two imperfect legs depending from the left buttock; but, as in the case of Louise L., there were no doubled parts in the autosite. This last fact, as well as the complete asymmetry of the parts, suggests that it is a case of parasite and autosite, although in many ways both this and the preceding are similar to Blanche Dumas and others cited under I, *h* and *i*.
4. Winslow's case. A girl of twelve, who died at the Hotel Dieu, in Paris, in 1733. A parasite female lower half hung from the left flank. Sensibility was common, *i. e.*, felt by the autosite.

c. Parasite attached to head of the autosite.

This is frequently in the form of a supernumerary head or merely a face, and may be attached to the side or back of the head, or in some other place. Home, 1790, figures a case in which the supernumerary head is attached by its vertex to the vertex of the autosite.[8]

A parasite attached to the jaw bone is termed an epignathus.

2. Parasite Developed Within the Autosite, usually in the Body Cavity, but Occasionally in Other Regions. These Form Tumors which usually Increase and have to be Removed by Operative Measures. Commonly Termed "Fetus in Fetu."

These cases, which it is not necessary to classify in detail, have been rather commonly reported for several hundred years; the parasite ranges in completeness from an almost perfect fetus to a shapeless mass containing bits of organized tissue, the so-called teratomata or dermoid cysts. Thus Blundell, 28-29, reports the case of "a boy who was literally and without evasion with child, for the fetus was contained in a sac communicating with the abdomen and was connected to the side of the cyst by a short umbilical cord; nor did the fetus make its appearance until the boy was eight or ten years old, when after much enlargement of pregnancy and subsequent flooding, the boy died." The parasitic fetus is, however, seldom as perfect as in the above but is more often quite rudimentary. In one case it consisted of "the ribs, the vertebral column, the lower extremities as far as the knees, and the two orbits"; in another "fetal bones and a mass of macerated embryo" and in another "hair, molar teeth, and other evidences of a fetus." In general such parasites show themselves in the autosite during the infancy or childhood of the latter, but, again, they may not appear until adolescence or even adult life. In location such enclosed parasites may be almost anywhere—the abdominal or thoracic cavities, the cranial cavity, the spinal canal, the scrotum, or urinary bladder.

ORIGIN OF COMPOSITE MONSTERS.

To one who sees in separate duplicate twins the result of the total separation of the first two blastomeres of a developing ovum there is but one rational explanation for diplopagi, or those composite monsters

[8] This case may have been that of *Craniopagi*, one component of which had suffered an early fetal amputation at the neck. See above, under *Craniopagi*.

in which the two components are the duplicates of one another[9] and symmetrically united, namely *that here a similar tendency to separation has been left incomplete, causing a doubling of those parts only in which the interrelations have been severed.* This hypothesis, which is the natural outcome of the recognition of these components as duplicates, finds its best corroboration in the fact that the cases may be arranged in graded series, with almost imperceptible differences between them, and ranging from single individuals with a duplication of some median part, to two individuals united by a slight bond, and thence to free duplicates. Diplopagi do not, however, allow themselves to be arranged in a single series, but in several, in which the two components bear different, *although always symmetrical,* relations to one another, referable to the various geometrical possibilities in the manner of separation and consequent relative position of the first two blastomeres.

This matter may be made clear by an inspection of Plate A, in which the various known forms of diplopagi are represented in diagrammatic form and arranged in graded series to show their mutual relationship.

These series and the cases representing them are as follows:

A.—Separation from Above Downward; also in Some Cases from Below Upward; Components Placed Laterally.

A. I.—A normal single individual, the right and left halves of which are represented by the colors black and white respectively. In the em-

[9] Although in the great majority of diplopagi the components are evidently physical duplicates, I have collected the three following cases in which differences seem to have been noted. (1) Paré's case, 1570, baptized Louis and Louise, (2) The Irish sisters, 1827 (v. p. 401), of whom one was said to be dark haired, the other fair, and (3) Marie-Rosa Drouin, 1878, one of whom was said by MacCallum to resemble the father and one the mother. Should those cases be substantiated they would necessitate a modification of my theory, at least in those special cases, but the reports are hardly conclusive enough to be of serious import. Paré's case was reported over three hundred years ago, and at a time when feminine names in France were assumed by men. There is no statement to the effect that they were of opposite sex. The other cases are based upon general impressions, in both cases derived from newborn infants, and hence are somewhat unreliable. *The most conclusive test to prove or disprove the similarity of the components, both in diplopagi and, when possible, in parasitic monsters, is that of the palm and sole configuration, and it is to be urged that, whenever opportunity affords, careful prints be taken of the palms, soles and fingers of compound monsters. Such data will prove of the highest importance in the present line of argument.*

bryonic development of this the first two blastomeres may be supposed to have shown the normal interrelation.

A. II.—A case of duplicity in certain of the parts of the face and head, resulting from a slight separation of the first two blastomeres in the region ultimately to become the anterior end of the embryo, after which, through the disturbed relations of these parts, each separate portion attempted to develop both sides.

Ex.—Moreau's case. Buffon's cat.

[Lesser degrees of doubling have been mentioned above in connection with the cases just cited.]

A. III.—Here the original separation was sufficient to involve the entire future head region, and each component blastomere, relieved of the normal stimulus of contact with the other upon one side, has regenerated the other half and thus developed an entire but duplicate head.

Ex.—The two-headed turtle of Barbour. Externally Gruber's "Case II" of Thoracogastrodidymus seems to belong here but the trunk is partially double. It forms the link between this and the next, as a little less separation would place it here, a little more in the next section.

A. IV.—This stage is the first in the series to show the significant phenomenon of a double median limb. In this and in all such cases the limb is a bilaterally symmetrical member, the two lateral surfaces being the same aspect (usually dorsal) and bearing the structures characteristic of the aspect presented. The structure bears so much the appearance of a fusion or coalescence of two originally separate components that many writers, perhaps the majority, speak of them as such. This latter view is, however, manifestly impossible, for, granting that two members, once separate, could ever unite in such perfect juxtaposition as to form a symmetrical piece, it would still be a serious problem to dispose of the surfaces in contact with each other, and to atrophy each component at such an equal rate that the result would keep the perfect symmetry which these phenomena show. In the diagram there is seen to be a doubled inner arm, contributed to equally by each component.

Ex.—To illustrate this case we are fortunate in having specimens, carefully described and in part dissected, which represent a graded series. Of these, the first is Gruber's "Thoracogastrodidymus I," which differs from his "Case II" of like name (A. III) merely in the presence of a small median rudiment containing a double scapula with a pair of conical acromion processes which together form the skeleton of the free end. Meckel's case possesses a larger median rudiment with a humerus

and an "os antebrachii," and in Barkow's fetus there were two radii, a median ulna, and a double hand with the full number of digits.

A. V.—A continuation of the same cause leads to a complete separation of the two inner arm-components, and allows each to develop as an entire organ. These inner arms are usually perfect in form but often show some defect of development which may easily be due to lack of space and the consequent cramped position in which they must be held. This stage is represented by a large number of cases, and is far more common than the intermediate stage represented by the preceding case.

Ex.—The Tocci brothers. Ritta-Christina.

A. VI.—This slight advance on the previous stage is characterized by a rudimentary median leg; otherwise it is like the last.

Ex.—Marie-Rosa Drouin.

A. VII.—In this the median limb has attained a considerable degree of development, this being, from the description of the single case recorded, of about the same grade as the arm in Barkow's case.

Ex.—Fisher's case, 1773.

Here the series ends, since there seem to be no known cases representing the next logical step, that in which the median double leg has separated into two, leaving two complete components, united by a small isthmus, laterally placed. Xiphopagous twins, like the Siamese brothers, often stretch the connecting bond so much that they can place themselves laterally, but the natural position of all such is face to face. The same is true to a lesser extent in pygopagous twins. It seems possible to consider that in this series a farther separation might affect either the ventral or the dorsal aspect of the body in such a way as to produce respectively either pygopagi or xiphopagi, in which case either would represent the missing stage, but in the present state of our knowledge this cannot be definitely assumed. Thus, in the diagram a space is left for conjoined twins laterally placed, and the series ends with the hypothetical case of separate twins laterally related (A. IX).

B.—Separation from Below Upwards; Components Placed Laterally.

B. I.—Here may be put individuals in which there is a greater or less doubling of the median pelvic parts, especially the external genitals. Such cases are rare but include both sexes.

Ex.—Several minor cases of duplicity of the external genitals, collected by Ballantyne and Skirving, 94-95.

B. II.—This is like stage IV of series A, with a median leg in place

of a median arm, and with a doubling of the pelvic organs in place of the head.

Ex.—Dos Santos. Bechlinger's case.

B. III.—In this there is a complete duplication of the body below the lumbar region, each half furnished with a normal pair of legs, although there may be some lack of development in the inner legs in the same way and for the same reason as in the case of the inner arms in V and VI of series A.

Ex.—Wells' case (Mrs. B.).

Beyond B. III there seem to be no authentic cases with laterally placed components, but the first stage of series C, although differing in the geometrical relation of the components, might in other respects stand as B. IV, in which, in addition to the doubling of pelvis and legs, there is an incipient doubling of the head.

Series C may thus be placed in the same row as B and may be designated as follows:

C.—Separation at First from Below Upwards, Succeeded by One from Above Downwards; Components Placed Face to Face.

C. I.—From the umbilical region downwards two separate bodies, chests united and each component less than a whole one; head partly doubled, with two occiputs, faces incomplete, with one median eye on each lateral aspect, formed of equal contributions from each component. An incomplete Janus monster.

Ex.—Fig. 5 a of this article, originally described by Förster, **61**, from the Göttingen collection.

C. II.—Typical Janus monster, like the last but with the two head components complete or nearly so. The laterally placed faces are in reality the left half of one face joined to the right half of the other, as explained above. Otherwise like C. I.

Ex.—Fig. 5 b of this article, after L. Blanc (I cannot find the original of this).

C. III.—Typical thoracopagi; they evidently belong in the series with the Janus monsters, from which they differ merely in the more complete separation from above downwards, thus allowing the development of two separate heads and necks.

Ex.—Siamese twins, Radica-Doodica.

C. IV.—Separate duplicate twins, like A. VIII, but differing geometrically in their mode of origin. This is probably the commonest type, if we may judge by the frequency of occurrence of thoracopagi.

D.—Separation from Above Downward, and None from Below Upwards; Components Placed Laterally.

This series has the first five stages in common with series A, and diverges from it at stage V (= D. I), beyond which A shows a separation from below upwards, *while D does not, but continues the initial separation from above downwards.* This results in (1) a greater and greater divergence of the body axes, and (2) the development of posterior limbs in the angle between them, forming ischiopagi. This method may ultimately result in separate twins as in the other series. The stages, after that corresponding to A. V, are as follows:

D. I.—This is practically the same as A. V (represented by the Toccis and Ritta-Christina), but with the separation a little farther down and the angle between the two trunks a little greater. The amount of divergence seems a variable quantity, and in fact the bodies are capable of some lateral movement. Thus, in the example of this, the "Monstrum anglicum" (Fig. 2, c) the bodies are represented in a contemporaneous engraving as almost forming the same horizontal line, but in the figure cited to represent the next stage (Fig. 2 a and b), the bodies form a very divergent V.

D. II.—The farther progress of the separation from above downwards has produced a double rudiment of a median leg, comparable to those of A. VI, B. II, and E; or to the double median arm of A. IV, E and F.

Ex.—Case figured by Förster, Tab. I, Fig. 15; after MacLauren, Phil. Trans., Vol. 32. Fig. 2 a and b of this article shows a slight advance beyond this.

D. III.—Stage with the upper posterior limbs, *i. e.,* those in the angle between the trunks, separate but rudimentary.

Ex.—An instance of this is given by Fisher, 66.

D. IV.—Typical ischiopagi, with limbs of both sides complete, and with perfect genitals and anus between them, formed by two halves contributed by the two components.

Ex.—Fig. 1 of this article; any typical ischiopagi.

D. V.—Here, as in other series, may be placed separate duplicate twins differing from the others merely in their mode of origin considered geometrically. These, like those resulting from series C, are probably a common type.

In the lower row of the diagram are collected a few more or less unique types of diplopagi, which show relationship to the several series above given, but which differ in extent of development of some part. They are as follows:

E.—Diplopagi Allied to Series A, but with the Separation from Below Upward Occurring Earlier than in the Regular Series. This results in the formation of a median double leg-rudiment in conjunction with a median double arm.

Ex.—Fenn's fetus, Fig. 4 a of this article.

F.—Ectopagi Allied to Series A and to E, but with the Separation from Below Upward Still Farther Advanced, giving each body a pair of perfect legs.

Ex.—Regnault's ectopage, Fig. 3 of this article.

G.—These are the Craniopagi, of which illustrations enough have been cited to form a series, were it not that they do not show much relation to one another. Thus, in G. St. Hilaire's case, G. I, the two components are united at their forehead, and in Villeneuve's case, G. II, the union is by the vertices, facing different ways.

It may be supposed in a general way that such craniopagi as G. I result from an extreme separation from below upwards, as in series B and C, perhaps as a continuation of C. II with more separation from below than from above; and that G. II may be related to it as the extreme of the ischiopagous series is to the rest; but in the absence of intermediate forms all this is a mere conjecture. The fact must be acknowledged that in the case of craniopagi we do not as yet possess sufficient data to form a series suggesting their origin.

Whether or not separate duplicate twins result from the craniopagous type cannot be certain, but it seems at least possible, and as such G. III is presented in the diagram. Such a type of twin, if ever formed, must be very rare, corresponding to the infrequency of conjoined craniopagi.

H.—Here, as H. I and H. II Respectively, are Placed Pygopagi, and the Free Duplicate Twins which may Theoretically Result from them.

As pointed out above, under series A, the origin of pyopagi cannot be determined, in spite of the frequency of the type, since all diplopagi which may be considered as incomplete pygopagi are thus far lacking. There is a temptation to put them in the missing place in series A (A. VIII), in which case there would be no such free twins as the laterally related ones figured as A. IX; but they seem to have no more right there than do the thoracopagi.

At present they are of as uncertain relationship as are the craniopagi, and although of frequent occurrence are best left in an isolated position in the diagram.

In the Above Sketch of the Interrelationships of Diplopagi it has been often Impossible not to Speak as though the various

FORMS WERE STAGES IN A CONTINUOUS DEVELOPMENT, AND ONE MUST BE ON HIS GUARD NOT TO LOOK UPON THEM AS SUCH. Instead of this they form the varying steps in a process in which each case is fixed, probably from the beginning, and represents but one step, and the process is continuous only in the sense that the various possible cases form a series of almost imperceptible gradation between two limits.

The hypothesis advanced here necessarily presupposes some form of preformation in the egg-cell and in the early blastomeres, but no more than must be granted by any one who studies the various correspondences found in separate duplicate twins, for example, the palm and sole configuration. The main factor, however, in the formation of either a diplopage or a duplicate twin is, however, *the changed relationship of the cells, a cell or a part of a cell developing differently when isolated than when in contact with another. A sea-urchin blastomere in the 2-celled stage, when in its normal relation to its fellow, develops but one side of a bilateral larva, but when this contact is severed, it develops both. Similarly, if the relation is lost in part and retained in part, a double monster is the result from the same cause.*

It is manifestly impossible thus to experiment with human eggs or with those of other placental mammals, but the results of observation indicate that what is true in the case of lower forms, obtains also here. In mammals the development of the extra-embryonal parts appears at first to greatly complicate the problem, and they certainly do introduce an element which cannot be wholly explained as yet in this connection, but the facts in the case are so numerous, and the relationship of the various forms of diplopagi are so simple and so completely analogous to the results of experiment among lower forms that the close similarity between the two cannot well be doubted.

There yet remains to be accounted for those cases of compound monsters in which the two components are very unequal, sustaining the relationship of parasite and autosite. These are plainly the result of a secondary fusion, probably at an early age, of two embryos developing in close proximity to one another, perhaps in the relation described in Schultze's Case IV. The components, in order to be in this relation to one another, must be originally duplicate twins, and as a matter of fact, in so far as the parasite possesses sex, it is always that of the autosite. It may be possible, of course, that under certain circumstances fraternal twins may fuse with one another, but the enclosure in separate chorions, which seems to be the rule, would normally prevent it.

As stated above, no tests seem ever to have been made in such cases relative to their original physical identity, although in many cases, such

as Laloo and Louise L., a comparison of palms and soles or of both, as applied in Part II to the case of separate twins, would be quite possible.

Separate duplicate twins, thus, have successfully avoided two dangers, (1) *that of an incomplete separation of the two blastomeres of the two-celled stage, and* (2) *that of a secondary fusion of the later blastomeres owing to close proximity; in the one case they would have become diplopagi, in the other, one would have been an autosite, the other a parasite.*

OTHER RECENT THEORIES CONCERNING THE GENESIS OF COMPOSITE MONSTERS.

The main principles along which theories on this subject, both ancient and modern, have developed, are the following: (1) the principle of excess and defect, (2) the principle of fusion, (3) the principle of fission, and (4) the principle of two original fetuses. These principles are for the most part extremely old and in their modern form differ from the ancient mainly in the more definite application made possible by the advance of knowledge of the subject. Thus where Empedocles spoke of an excess of semen, and Democritus of two "seeds" we now speak definitely of polyspermy; the theory of Aristotle, who sought for the origin of such monsters in an excess of material, was the precursor of the theory which postulates a doubling of the determinants in the germ-plasm. Another characteristic of modern theories is that they often make use of two or more of the above principles in combination, although the supporters of the various principles seldom, if ever, agree upon the method of combining them, or in the precise method of their application.

Since almost every teratologist who describes a case develops a theory concerning its origin, it is manifestly impossible here to attempt a digest of all the recent work in this line, but at the risk of unwittingly omitting many valuable scientific contributions to the subject I will present here what seem to be the principal theories of the present time, selecting as their exponents those authors who have treated the subject in a general way and who have familiarized themselves with a large number of cases.

I.—The first of the modern theories to be considered postulates AN EARLY TOTAL FISSION OF THE EMBRYO, *followed in the case of compound monsters by a secondary fusion of the two parts.* Of this school the pioneer seems to have been Fisher, 66, who, although writing nearly forty years ago, anticipated many of our most advanced ideas on the subject. He says that double monsters "are invariably the product of a single ovum, with a single vitellus and vitelline membrane, upon which a double cicatricula, or two primitive traces, are developed. The several

forms of double malformation, the degree of duplicity, the character and extent of the fusion, all result from the proximity and relative positions of the neural axes of two more or less definite primitive traces developed on the vitelline membrane of a single ovum." In this, by the supposition of a "double cicatricula," he implies, to us at the present time, a fission of the original germ, but, although he comes fairly up to this point, he seems to have nowhere definitely expressed as much and such a conception was clearly beyond him at that date. The "double cicatricula" being present, he supposes the monster to be formed by a subsequent fusion of the "primitive traces." In true double monsters he recognizes the following characters: (1) "homologous union" (*i. e.*, a symmetrical position of the two components); (2) unity of sex, and (3) the transposition of viscera in one of the components, a condition the necessity of which he somewhat doubts, although he gives several instances of it.

The step that Fisher did not take, that of accounting for the "double cicatricula" in the first place by a fission of a single germ at an early period, was proposed soon after the date of his paper, and probably originated with Dareste, although the idea seems to have been evolved independently by Galton and others at about the same time. Dareste, however, was working upon the subject of double monsters and Galton upon that of duplicate twins, and it seems to have been first applied to both by Perls, **79,** who starts in both cases with the fission of an originally single germ, the two resulting parts of which normally become two separate individuals, but which may, in rare cases, fuse secondarily into a compound monster. Thus Blanc, **93,** describes the origin of ischiopagi as follows: "Les monstres ischiopages résultent, ainsi que l'a montré M. Dareste, de la soudure de deux embryons, opposés par leur extrémité posterieure et placés dans le prolongement l'un de l'autre" (p. 238). He then explains that if the two embryos fuse along a straight line the legs and pelvic organs have room to develop "déviés à droite et à gauche," but that if they form an angle with one another the parts enclosed by this angle suffer, owing to lack of space.

Although not quoted directly from Dareste himself, it is altogether probable that the above clearly represents his views, at least concerning one type of diplopage. The same view is held by his associate, Baudouin, **91,** who states that in the production of diplopagi the two primitive streaks unite secondarily at a given angle with one another, and that the variations depend upon (1) the precise epoch at which the fission takes place, and (2) the size of the angle formed by the two axes.

The various arrangement of these axes may be in the form of an upright Y, an inverted ʌ or an X, thus forming three convenient classes.

Kaestner, **98**, throws some light on the subject by describing a few actual cases, among them that of a shark's egg (Pristiurus), which had two germinal discs. As the egg was transparent the development could be watched, and it was observed that one of these discs soon surpassed the other in size, and that the latter became adherent to the former by the margin. He reasons from this that only in mammals, because of the rudimentary character of the yolk sac, can two such blastoderms ever hope to develop as separate individuals, but that in others (fishes, reptiles and birds) the common yolk must lead eventually to a fusion in all cases.

It will be seen that the principle common to all of the above theories is that of the secondary fusion of two germs, but *an insuperable objection to this lies in the complete bilateral symmetry of the two components in true double monsters (diplopagi), since there is no force to oversee and adjust the two components in the exact relationship necessary for this result.* That such a fusion may result in the other type of compound monster, that of autosite and parasite, seems most probable, a result to which, in all likelihood, the Pristiurus egg of Kaestner would have attained, had it completed its development.

II.—A second class of theories rests upon the supposition of A PARTIAL FISSION IN A SOMEWHAT ADVANCED EMBRYO, thus doubling the parts affected, and several writers have attempted to prove this principle by practical experiment. Thus Valentine, **77**, supposes that he produced a double chick by artificially splitting the "Keim," and Gerlach, **83**, produced a few very questionable specimens by varnishing the shells of hens' eggs and leaving an unvarnished space in the form of a V or a Y over the blastoderm.

III.—The RADIATION THEORY of Rauber is somewhat fantastic and difficult to comprehend, and possesses in this connection mainly an historic value. He postulates in the first place *a tendency of the germ-cells to radiate in all directions,* to assume "eine strahlige Anordnung," a tendency which leads occasionally to the formation of a "Keimring mit mehreren vorderen Embryonalanlagen, statt wie gewöhnlich, einer einzigen." The bifurcation of the anterior end, as taught by Gerlach, Rauber considers a modified form of radiation, and speaks of a *radiatio anterior* and a *radiatio posterior.* This theory, like those of Valentine and Gerlach, supposes a splitting of a somewhat advanced embryo, but adds a possible, although very hypothetical, cause.

IV.—Under the head of SUPERFLUITY (the "monstra in excessu" of the older writers) may be grouped the theories of several investigators that see a cause for compound monsters in an excess of some of the elements of the original germ. Thus O. and R. Hertwig, having suggested as a working hypothesis that such cases are the result of polyspermy, attempted to develop double monsters from the eggs of sea-urchins into which more than one spermatozoon had been introduced. The results in this case were wholly negative.

Wiedemann, **94**, finds the superfluous material in both the male and female elements and believes that compound monsters arise from the presence of two germinal vesicles on a single yolk, each fertilized by a separate spermatozoon. The great irritation produced by the two spermatozoa on the single egg occasions contractions which tend to draw the two vesicles together. When there is protoplasm enough for two good blastoderms they remain separate, and produce "die sogennante eineiige Zwillinge;" when not, a compound monster is the result.

Considerable apparent support has been given to theories like the foregoing by the discovery in the ovaries of various mammals, and recently in man also, of primordial eggs with two nuclei, and of follicles enclosing two eggs, and an interesting discussion was begun by v. Franqué, **98**, and taken up by others (see Bibliography, IV), with the final conclusion that the mature result of such phenomena is always two separate eggs, although they may be discharged at one time, even from the same Graafian follicle. There seems to be no reason why those eggs should produce compound monsters, or even duplicate twins, since they must be fertilized by different spermatozoa, but that fraternal twins may often, if not always, result in this way seems probable.

A theory which is in some ways the counterpart of that of a double egg is the one recently suggested by Broman, **02**, and based upon the occurrence of various forms of double and otherwise compound ("atypische") spermatozoa in man. Of these he figures a large number of forms and comes to the conclusion that "die zweischwänzigen Spermien für die Entstehung eineiiger Zwillinge wahrscheinlich eine grosse Rolle spielen können. In derselben Weise können wahrscheinlich die drei- oder vierschwänzigen Spermien zu eineiigen Drillingen resp. eineiigen Vierlingen Anlass geben" (*loc. cit.*, p. 526).

This theory he extends to double monsters because of the existence of all transitional stages and concludes, if we accept the above conclusions "dass wir auch fur die Genese der Doppelmonstra den atypischen Spermien eine mögliche Bedeutung zuerkennen müssen."

Another form of the theory of superfluity is that of Windle, 93, who, quoting Sutton, compares the production of a double monster to "the same tendency which causes dichotomy of the ray in star-fish, or digits in mammals, a tendency which, should it affect the axis of the embryo, will lead to the production of duplex monsters of varying development and different degrees of union, or even result in viable twins." He adds: "This statement, so far as it goes, concisely states the view which I hold on the subject." In his summary he gives as the ultimate cause for this dichotomy a superfluity of germ-plasm existing in the germ." This superfluity may be the result of (1) a faulty segmentation of the polar bodies, (2) a faulty formation of the spermatozoon, or (3) polyspermy. His statement that this superfluity in true double monsters leads to a fission "prior to that by which normal development is commenced" is practically an expression of the theory of the partial or total fission of the first two blastomeres, as advocated in the present paper, with the interesting addition of a possible cause for the phenomenon. He causes some confusion, however, in attempting to bring into the same category all cases of minor duplicity, such as hyperdactylism, and by asserting that there is "no gap" between such cases and true double monsters.

V.—As representative of the FUSION THEORY, by which two original embryos become a composite monster by atrophy of certain parts, we may cite Panum, who holds that *all cases involving the doubling of trunk and limbs presuppose two fetuses,* originally with a completely or partially double axial anlage, one-half of which has become atrophied during the course of development. He acknowledges, however, that many cases of minor duplicity may be the result of budding.

VI.—A unique theory is that of POSTREGENERATION, recently brought forward by Tornier, 97-01, to explain cases of minor duplicity and later extended so as to include all cases of compound monsters. Tornier has studied experimentally certain of the well-known cases of regeneration found among lower animals, such as the tails of lizards, the limbs of salamanders, etc., and has shown that while a complete loss of the member will result in a simple regeneration of the part, yet that a certain kind of wound, made in the place where the loss usually occurs, yet insufficient to cause the loss of the part, will occasion the growth of a new member in the same manner as though the original had been actually lost, thus producing duplicity. From this he holds that, while such phenomena may occur after birth in certain of the lower animals only, they yet may occur before birth, in birds and mammals, as a result of a lesion affecting the fetus. This seems a reasonable theory when con-

fined to such cases as hyperdactylism, but Tornier in a later paper, **01**, wishes to go much farther than that and asserts that all cases of double monsters (Zwillingsbildungen) arise in the same manner, and cites, to illustrate his meaning, not only individuals with two heads, and those with two faces (the so-called "Janus-monsters"), but also typical thoracopagi. As this theory presupposes an inequality in the value of the two components, since one must be the "Stammindividuum" from which the second or "Ueberzähliges Individuum" must arise secondarily, it is hard to reconcile with it the exact equality in the development of the two components in the overwhelming majority of cases; and as separate twins of the duplicate type are so closely related to the most complete double monsters, they also must be included in the theory, a supposition apt to give rise to endless dispute among two people thus related as to which was the "Stamm-" and which the "Ueberzähliges-Individuum."

VII.—A most unique theory is that of Beard, which he develops in connection with his original biological doctrine of MULTIPLE GERM-CELLS. He considers that the early blastomeres of a metazoon form "an asexual foundation or larva, the *phorozoon*, upon which the germ-cells, and with those an embryo, take their origin." Normally one of these cells develops into an embryo, a mere incident in the life-cycle, but occasionally two or more may thus develop. Thus "if two primary germ-cells undergo normal development, the result is the production of identical twins." An attempt on the part of two germ-cells thus to develop but with abnormalities on the part of one produces a more or less rudimentary embryo, which may form any grade of morbid growth from an embryoma to an ordinary tumor or cancer. Wilms has conclusively demonstrated that, for example, all gradations from a highly-organized embryoma down to a simple sarcoma may be met with. In this discussion Beard plainly has in mind the various forms of parasitic monsters alone, since in all of his descriptions of compound forms one component is normal and the other reduced. There is no doubt that, granting his theory in the first place, there would be some logical explanation also for diplopagi, and it will be of interest to see what this author will do with this most important group of composite monsters.

VIII.—As opposed, at least in some particulars, to all of the above hypotheses is that which sees the cause, both of separate duplicate twins and of diplopagi, in the TOTAL OR PARTIAL SEPARATION OF EARLY BLASTOMERES, PROBABLY OF THE FIRST TWO, as advocated in the present paper. This view as extended to diplopagi seems to have been first clearly enunciated by Bateson, **94**, who says: "It is now a matter of

common knowledge that in animals (and plants) division may occur in such a way that two or more bodies may be formed from what is ostensibly one fertilized ovum. But *by a similar division, imperfectly effected, the resulting bodies, instead of being complete twins or triplets, may remain united together, frequently having a greater or less extent of body in common.*" The italics are my own, and point out with extreme clearness my views on the subject as explained above. Bateson, however, continuing farther in his ideas, and believing that the composite body must show a complete bilateral symmetry, assumes, as Fisher did, the need of a *situs viscerum inversum* in one of the components, a view which appears to be upheld in a few instances[10] but fails in others. On this point he finally concludes that the amount of transposition depends upon the time of separation, a view the precise meaning of which is not clear in connection with his former explicit statement that such cases arise from the division of "what is ostensibly one fertilized ovum," and suggests an unwillingness to abide by his theory so clearly expressed above.

More recently this theory has been advocated by Sobotta, 00,[11] although he is uncertain at what point in early development the separation takes place. As opposed to the belief that this point is that of the two-celled stage, he suggests (1) that we do not know whether or not these blastomeres in the human species are "äquipotent;" (2) that it is difficult to conceive of a force capable of separating these blastomeres that lie in one egg, protected by the zona pellucida, the oviduct, etc.; (3) that if one supposes a total separation, as in "eineiigen Zwillingen," it is hard to understand how the chorion came to be single, as seems always to be the case in such instances. He does not seem to consider these objections fatal, however, and although he inclines to the belief that the separation takes place during the later cleavage or in the "Keimblasenstadium," he admits that "die Ursache der Doppelbildungen auf einem früheren Stadium zu suchen ist als im ersten Augenblick, wo sie der directen Beobachtung zugänglich [sind]." On the other hand he says: "Vielleicht kann auch bei stark verwachsenen Doppelbildung die Ursache der Störung noch viel später einwirken auf die Area embryonalis selbst und *selbst*

[10] For this Bateson quotes Eichwald who finds that in thoracopagi "in almost every case one of the bodies shewed some transposition of viscera, though to a varying extent." (Pet. Med. Zeitsch., 1870, No. 2.)

[11] The original paper of Sobottta has unfortunately not been accessible to me, and I have relied entirely upon the review of the same given by Broman, 02. I hope that, in spite of this disadvantage, his views have been fairly represented.

auf den schon im Entstehen begriffenen Embryo." The italics are my own and show that, in spite of what seems to be his inclination, Sobotta can hardly be included in this section but belongs rather more to the school of Valentine and Gerlach, who advance the period at which the splitting takes place to some point considerably beyond that of the early cleavage stages (II).

The authors cited above are a small number of those who have expressed their views upon the subject, but it is hoped that the exposition of theories includes practically all that are held upon the subject at the present time. For farther information the reader may consult the bibliography at the end of the paper, especially the papers of Wiedemann and Windle, who enter into the history of the subject in some detail, and also the general works on the subject.

PART II.

STUDIES OF THE CONFIGURATION OF THE FRICTION-SKIN[1] (PALMS AND SOLES) IN TWINS AND TRIPLETS.

Scope of the Investigation.

The physical "identity" of duplicate twins, in so far as it has been considered at all, has hitherto rested upon such data as facial expression, color of hair and eyes, and physical proportions; features which, although perfectly reliable as far as they go, lack the definiteness necessary in a scientific comparison.

Facial expression is often strikingly similar in two brothers or two sisters of separate birth, and, on the other hand, genuine duplicate twins may be subjected during life to circumstances that differentiate them to a marked degree. The same may be said of bodily measurement, even those included in the Bertillon system, since, as will be shown, the

[1] The term "friction-skin" was proposed by me and used in a paper now in press, written by my pupil, Miss Whipple. It designates that modified form of skin found upon the contact surfaces of the ventral aspect of mammalian chiridia (hands and feet), which consists of ridges placed at right angles to the direction of the most usual forces and designed to prevent slipping after the manner of the milling or grooving in the handles of certain instruments. The ridges themselves, which in the higher Primates cover the entire ventral surface of the hands and feet, are called in the paper referred to "friction-ridges," a term properly expressing their true function, and hence not misleading as is the term "papillary ridges" hitherto used. A full description of these terms and of the physiological action of the parts involved will be found in Miss Whipple's paper.

slightly different external circumstances of nutrition, activity, etc., will develop differences not present at first and not directly connected with the question of the original physical identity. In the study of the epidermic ridges of the ventral surfaces of hands and feet, however, we have an exact field for research, since there is a set of characters here present which are laid down in the embryo and persist until death, beyond the influence of any external change. They are, moreover, actual anatomical parts which can be studied and described, counted and compared, and reproduced for illustration by the exact method of printed impressions, a method by which each reader can have the data placed before him as accurately as though he could investigate the objects himself, and in a much more convenient form.

Galton, **92** (pp. 185-187), seems to have been the first to make use of these parts in the study of twins, but he confined his examinations to the apical patterns (finger-tips) alone, and although he was one of the first to formulate a scientific distinction between the two types of twins, yet in these investigations he makes no distinction between them, undoubtedly because in this place his main object was to show their differences and not their similarities. That he then, however, had in mind a more careful biological investigation of exactly this point is shown by the following statement, which is added to the investigations referred to, and which is so exactly the object of a part of the investigation of this paper, that I only hope that my plans, worked out quite independently, and at first without the knowledge of this statement, may not in any way interfere with anything he may have been preparing. He says: "It may be mentioned that I have an inquiry in view, which has not yet been fairly begun, owing to the want of sufficient data, namely, to determine *the minutest biological unit that may be hereditarily transmissible.* The minutiæ in the finger-prints of twins seem suitable objects for this purpose."[2] (The italics are my own.)

Since Galton, in the work cited, made no direct use of the epidermic markings in the study of duplicate twins as distinguished from the other types, and since he does not seem as yet to have published any investigation along the line of his statement quoted above, it appears that I have been the first to actually undertake such an inquiry. In my article "Palms and Soles" (Amer. Jour. Anat., 1902) I have published reduced tracings (not prints) of the full set of palms and soles of a pair of duplicate twins as well as the hands of a second pair, and have shown the great similarity of those parts, "which are naturally greater than

[2] "Finger-Prints," 1892, p. 187.

could ever exist in any other two people" (p. 435). Although these tracings and the brief statements accompanying them gave a general idea of the remarkable correspondences in such cases, they formed scarcely more than an announcement of an interesting line of investigation, and it is to supplement this that the facts presented in the present paper are given. In this the first consideration will naturally be bestowed upon the epidermic markings, although in order to make the investigation as complete as possible, I have thought it advisable to add anthropometric data and other matters of interest.

The material at my disposal for use in the present investigation includes some data, at least, and in nearly all cases sets of prints, of 16 sets of twins and two sets of triplets, as shown in the following table:

The sets are designated by Roman numerals and the individuals of a set by the letters x and y (and z), both sets of designations corresponding to names and numbers as listed in my private collection. In the determinations of the physical appearance I have designated with an asterisk all cases that I have had the opportunity of observing personally. In the other cases I have depended upon the descriptions of others, usually the collectors of the prints. In the most of the "unlike" cases the difference is so marked as to leave no doubt as to the determination, notably in the case of No. VIII, where the two individuals, although both of the same sex, are strikingly unlike in every way, while a third sister, two years younger than they, resembles one of them almost enough to pass for her duplicate. Nos. VII and X are the only ones that occasioned any hesitation and in this I was prejudiced by statements of the families, who, in both cases, consider that the twins are very much alike, but the differences seen by unprejudiced observers are sufficient to class No. X positively, and No. VII probably, in the fraternal class.

I may here take the opportunity of thanking the numerous persons who have aided me in this investigation; several of my present and former pupils, to whom I am indebted for Numbers I, II, III, IV, XVI, and XVIII; my assistant, Miss Inez Whipple, who obtained the data of No. VII and the photograph of No. XVIII; Miss L. I. Mattoon, who collected Nos. XIII and XIV; Miss C. F. Robinson, who collected the valuable set of triplets, No. XII; and Dr. R. H. Johnson, of the University of Wisconsin, who sent me No. V. I wish also to thank Prof. Edward Hitchcock, Dean of Amherst College and Director of the Gymnasium of that place, and Miss Senda Berenson, Director of the Gymnasium of Smith College, who have presented me with the anthropometric measurements made use of here. My thanks also would not

TABLE I.—LIST OF MATERIAL FOR THE PRESENT INVESTIGATION.

No. of set.	Sex.	Age (approximately).	Degree of physical resemblance.	Data in my possession.
I.	Female.	22	Identical.*	Palm and sole prints. Rolled finger prints. Anthropometric measurements.
II.	Female.	22	Identical.*	Palm and sole prints (right foot of X not taken). Anthropometric measurements.
III.	Female.	20	Identical.	Palm and sole prints. Anthropometric measurements.
IV.	Female.	20	Unlike.	Palm and sole prints.
V.	Male.	Not ascertained (adult).	Identical.	Palm prints.
VI.	Male.	17	Identical.*	Anthropometric measurements.
VII.	Female.	15	Considered alike, but with a difference in height of one inch; and otherwise not identical; probably fraternal.*	Palm and sole prints. Rolled finger prints. Rolled toe prints. Bertillon measurements.
VIII.	Female.	21	Very unlike.*	Palm prints Rolled finger prints.
IX.	Male.	15	Identical.*	Palm prints. Rolled finger prints.
X.	Female.	10	Similar but not identical; differ a little in height and weight.*	Palm prints. Rolled finger prints.
XI.	Female.	20	Identical.	Palm prints (very poor).
XII. Triplets.	Two males. One female.	4	Two males identical; female, "some resemblance to the others, but not much."—C. F. R.	Palm and sole prints. Rolled finger prints.
XIII.	Female.	25	Identical.	Palm prints. Rolled finger prints.
XIV.	Male.	7	Identical.	Palm prints. Rolled finger prints.
XV.	Female.	20	Identical.	Palm and sole prints. Rolled finger prints.
XVI.	Female.	16	Unlike.	Palm prints.
XVII.	One male. One female.	12	Unlike.	Palm prints.
XVIII. Triplets.	Female.	(Adult.)	All identical.	Photograph and written description.

be complete without including the many twins and triplets themselves, who have in all cases submitted to the ordeal of being printed with exemplary patience, and who in some cases (Nos. XI and XV) have taken the prints themselves.

EXPLANATION OF DESCRIPTIVE FORMULÆ.

The method of formulating the arrangement of the friction ridges of the palm, as given in my previous articles on the subject, and especially in the last one (Popular Sci. Monthly, Sept., 1903), makes it possible to easily describe and compare the large amount of material in the collection listed above. In preparing this description my object has been to formulate each palm without prejudice, losing sight, so far as possible, of the degree of similarity between the individuals of each set.

After going through them in this way, however, I have compared them with the previous decisions as to identity, and am willing to confess that I have occasionally added an explanation in those cases in which the formulæ fail to suggest the close similarity that is actually present, marking such additions in all cases by placing them between brackets. That this is occasionally necessary may be acknowledged by imagining the not infrequent case of two main lines which pass in opposite directions very near one another, and where the variation of a single ridge may cause either of the two to be the upper line, or may cause them to meet and fuse. If we imagine, for example, lines C and D in such mutual relations as these, the results expressed in formulæ would give for the first two figures either 7.5 (with line D above), 9.7 (with line C above), or, finally, 8.6 (with a fusion of the two lines in question), a difference of formulation which is very misleading when we consider that these three conditions may be almost exact equivalents of one another.

The formulations here given, aided by an occasional explanation in brackets, will furnish a brief but concise and definite description of all the palms listed above; and a comparison of this with the classification based upon the facial resemblance and general appearance will easily show whether the correspondence between the two, as demanded by theory, be warranted or not. As explained in the article referred to, each formula consists of four numbers, which designate the termini, and hence the mutual relationships, of the *four main lines* (the "primary" lines of the 1902 articles) in the reverse order, beginning with line D. The significance of the various numbers is shown by the diagram (Fig. 6) reprinted from the Popular Science Monthly with the kind permission

of the editor, Prof. J. McK. Cattell. The capital letter to the left of the four numbers designates the condition of the carpal region, whether a *carpal triradius* (C) is present, a *parting* (P), or a *seam* along the inner edge of the hypothenar region (S). The second row of designations refers to the presence and the conditions of the various patterns, and will explain itself. I have added also, in all cases possible, the formulæ for the finger-print patterns after the Galton system. In all cases the upper row of designations or formulæ are those of the individual catalogued in my list as x, the lower row those of y. The designations of the finger-prints (but not those of the main lines) are arranged in the order in which they would naturally come were the reader to lay his hands, palm down, on the table before him.

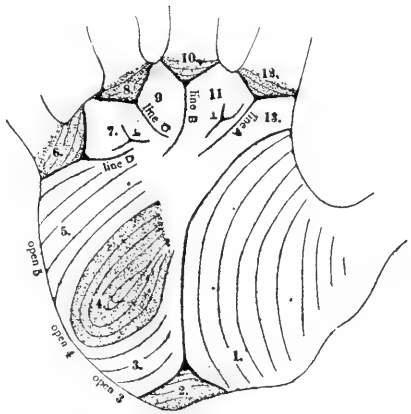

FIG. 6. Diagram of a left palm, showing the designations employed in making the descriptive formulæ.
[From Pop. Sci. Monthly, September, 1903, by permission.]

To make this plain, the descriptive formulæ of No. XVI are placed here as an example, and the significance of each designation explained.

No. XVI.—PALMS.

Designations.	Left.	Right.
Main lines, etc.	D C B A Carpus.	D C B A Carpus.
Their termination in x.	10 8 6 5 C.	11 9 7 5 S.
Special features.	Line C wholly wanting.	A carpal triradius high up in seam.
Their termination in y.	7 5 5 1 S.	9 7 5 5 S.
Special features.	A carpal triradius high up in seam.	A carpal triradius high up in seam.
Remarks on special features, resemblance, etc., in brackets.	[The marked differences in the formulæ diagnose these at once as twins of the fraternal type, a decision that receives corroboration from the general aspect and facial expression. This set was sent in by a student with no statement concerning resemblance, but on inquiry subsequent to making out the above formulæ, I found out that the twins were very different from one another.]	

No. XVI.—PALMS (*continued*).

	Finger patterns.									
Number of finger.	5	4	3	2	1	1	2	3	4	5
Finger patterns in x.	u	u	u	w	u	u	w	w	u	u
Finger patterns in y.	u	u	u	u	u	u	u	u	u	u
Remarks on the finger-patterns, in brackets.	[These also show a marked difference. The prints of y are all of the simple type of ulnar loops, with some variation in their cores, as usual. In x there are several whorls.]									

In describing the other sets the designations will be arranged in the same relative positions, but the interpretations will be omitted.

DESCRIPTIVE FORMULÆ OF THE PALMS AND FINGERS.

The list of descriptive formulæ is as follows:

No. I.

11. 7. 7. 2. S	11. 7. 7. 2. S
11. 7. 7. 2. S	11. 7. 7. 2. S

[Notice in these palms the complete bilateral symmetry between those of the same individual. The "S" is a new designation, and expresses the condition in which there is neither carpal triradius nor a definite parting of the lines at the wrist, but a seam or break in the direction of consecutive lines, which runs up across the palm, defining the hypothenar area and forming the only element upon which to base the carpal line. The left palms of this set are figured in Fig. 7 and are made the subject of special study later on in the text.]

u u u w u	w u u w u
u u u w u	u r u w u

[Note the reversal of the pattern of the right index, an ulnar fold in one and a radial in the other. Note a similar peculiarity in either the left or right index of many other duplicate twins, and see the comments below on this point.]

No. II.

7. 5. 5. 5. P	8. 6. 5. 5. P
H single broad loop.	H round loop, rather low.
8. 6. 5. 5. P	8. 6. 5. 5. P
H single broad loop.	H round loop, rather low.

[These are remarkably similar, and correspond exactly in many little details not apparent in the formulæ, as in the general appearance of the hypothenar patterns, the loops enclosed by lines C and D, etc. The single lack of correspondence, that of the first two figures of the left hands, does not alter the general configuration and in fact I am not sure but what they should really read the same, since the critical spots are covered up by broad red pencil lines, placed there before I realized the value of keeping the original prints unmarked.

Were the "7.5" of x changed to 8.6 as may well be possible, we would have another case of the complete bilateral symmetry in the hands of each twin as in the case of No. I.]

u u u a^r u	u u u w u
u u u a^u u	u u u w u

[Here the patterns of the left index are reversed, although it is not noticeable at first, since the pattern in question belongs to the arch type, normally bilaterally symmetrical. By a careful examination, however, the arch is seen in each case to bear a small loop along one side, the loop being *radial* in one and *ulnar* in the other. This I have indicated by the little letters used as exponents.]

No. III.

11. 9. 7. 5. S	12. 10. 8. 6. S
Small θ.	θ in form of a double curve.
11. ^{o} ` 5. S	12. 10. 8. 6. S
Small θ.	θ in form of a double curve.

[Reduced tracings of these palms were published in Amer. Jour. of Anat., Vol. I., pp. 436-437, and show their complete identity. While in one way no nearer alike than are the two preceding sets, there are more special features to be copied and the duplication is thus very complete, and more striking. The twins themselves, who are young ladies of twenty, had long noticed the curious patch of cross ridges on their left palms which represents a rudimentary "thenar," or morphologically a first interdigital.]

u w w r u	w r w w w
u w w r u	w r w w w

[There is here no reversal of the pattern in either set of indices.]

No. IV.

$\perp^3$(11). 8. 7. 3. S(=C ^{high}).	11. 8. 5. 5. S
No H.	No H.
11. 9. 7. 3. P	11. 9. 7. 5. S
No H.	H small loop, low.

The palms represented by these formulæ are very unlike in general appearance. The high position of C in x-left gives to that hand an especial character as that condition always does; and shows a marked contrast to the condition of the corresponding palm of y. The twins of this set do not at all resemble one another and are undoubtedly fraternal.]

u w w a u	u a u w u
u w u r u	u u u w u

No. V.

8. 6. 5. 3. C	8. 6. 5. 5. C
No H.	No H.
$\perp^3$(8). $\perp^3$(6). 5. 3. C	8. 6. 5. 5. C
H small loop, very low.	No H.

[These are practically alike, with two exceptions in the left hands, which are of slight morphological value. In *y* there is a third lower triradius ($\perp^3$), through which the last two lines (C and D) run. In order to indicate this, quite a change in the formula is necessary, but it will be seen that the final courses of the lines in question, as indicated by the numbers in parentheses, are the same as in the case of *x*, *i. e.*, 8 and 6. The small loop in *y*, classed as an H pattern, is very rudimentary. In all other respects the palms are exact duplicates.]

u u u u u	u r u u u
u u u r u	u r u u u

[Again the same inexplicable reversal of the pattern of one of the index fingers, this time the left, an *ulnar* loop in one case and a *radial* in the other. Compare I and II.]

No. VI.

[Of this set I have the statistics alone. I have met these twins personally and the resemblance is wonderfully exact.]

No. VII.

11. 8. 7. 2. S	11. 7. 7. 2. S
Line C ends in loop.	H almost rudimentary.
H complete with three triradii.	A wide open loop.
9. 8. 11. 3. C	9. 7. 5. 4/5. C
Line C ends in loop.	H a closed loop; lower inner tri-
H a simple loop.	radius present.

[This case has caused me considerable trouble, owing to a preconceived notion that the marks ought to be found identical. The family emphasized the facial resemblance of these twins and when I first saw them they certainly looked much alike. One was, however, an inch taller than the other, and the facial resemblance, after a short acquaintance, did not seem as great. Upon an unprejudiced comparison the prints of the palms are very different, and not at all as in the case of true duplicates. The finger patterns also do not at all correspond. The sole markings are similar, but not identical. The case is plainly one of fraternal twins that resemble one another somewhat more than the average.]

u u u ar u	u u u w u
u u u u u	w a u u u

[No correspondence here.]

No. VIII.

$\perp^3$(11). 9. 7. 5. C	7. 9. 7. 5. C
6. 8. 5. 5. C	6. 8. 5. 3. C
u u u u u	u u u u u
u u u r u	u w u u u

[These are the fraternal twins referred to above, which are extremely unlike, the one tall and slender, and the other somewhat shorter and quite stout. The latter so closely resembles a sister two years younger that they cause some confusion to those who do not know them well.]

No. IX.

9. 7. 5. 3. C	10. 9. 6. 3. C
9. 7. 5. 3. C	10. 9. 6. 3. P(?)

[As may be supposed, these boys are in every way identical. The slight discrepancy in the right carpal formula, a triradius (C) in x and a parting (P) in y is very likely an error, since in a print one cannot distinguish between a genuine parting and the lines which diverge to form a triradius that occurs below the limit of a print. In fact it is a question whether or not all genuine partings are to be interpreted as the beginnings of "extra-limital" triradii, or those which would be formed by the imaginary continuation of the ridges beyond the limits of the friction skin. Neither palm possesses any special features other than those indicated in the formulæ.]

u u u r u	u r a w u
u u u r u	u r r w u

[There is here no reversal in either set of indices, but the right hands show a difference in the pattern of the middle finger, a condition not seen in any of the other cases of genuine duplicates.]

No. X.

8. 6. 5. 3. C	10. 9. 6. 3. C
$\perp^3$. H narrow loop.	
7. 5. 5. 3. C	9. 7. 5. 4/4. P(?)
	H closed loop.

[These twins caused me some little difficulty, although they show by the formulæ great differences and determine the set as fraternal beyond a doubt. The subjects are little girls of ten, whom I have seen but once, and at the time I took it for granted that they were duplicates, and, as they came to my laboratory hand in hand, dressed exactly alike and each with her hair in two small braids; they were certainly similar, but to my assistant they did not appeal in the same way, and she judged them fraternal before seeing the prints. There is a noticeable difference in height and quite a little in weight, greater than is usually found in true duplicates.]

w w w w ?	? w w w w
w w w w ?	? u u w w

[The finger patterns of both are mainly of the whorl type, but even here y is at variance with her sister in two digits, which possess ulnar loops.]

No. XI.

11. 9. 7. 5. C

A curious loop on 4th interdigital area, perhaps accounted for as an inverted $\perp^3$.

11. 9. 7. 5. C

The curious loop, identical with that of x-left.

11. 9. 7. 5. C

A rudimentary $\perp^3$, which forms a lenticular figure, made by a parting of the ridges.

11. 9. 7. 5. C

A rudimentary $\perp^3$, forming an exact duplicate of the figure of x-right.

[These furnish another case of undoubted duplicates, and the prints would be especially fitted for illustration in this paper, were it not for the fact that they were taken by the twins themselves before receiving proper instruction and are barely sufficient for study, still less for printing. It is also unpractical to attempt to procure others in this case. Note the complete symmetry of the two sides in both, as in many of the cases of duplicates, although occasionally found in other individuals.]

u u u u u	? u u u u
u u u u ?	? u u u u

[The finger patterns seem to be all ulnar loops, with no reversal of index patterns.]

No. XII (Triplets).

$\perp^4$(11) 9. 7. 5. C	11. 9. 7. $\perp^1$(5)
$\perp^1$ encloses a pattern. Rudiments of H and θ.	$\perp^3$ encloses a pattern. Rudiments of H and θ.
7[$\perp^3$(11)] 9. 7. 5. C	11. 9. 7. $\perp^1$(5)
$\perp^1$ encloses a pattern. Rudiments of H and θ.	$\perp^3$ encloses a pattern. Rudiments of H and θ.
10. 7. 6. 5. P	11. 9. 7. 5.
$\perp^3$ encloses a pattern. Rudiments of H.	Neither $\perp'$ nor $\perp^3$ rudiment of H.

[These triplets are made the subject of a detailed study elsewhere and the palm prints are represented in fac-simile in Figs. 8 and 9. It will be seen from these that the difference in the first figure of the formula for the left hands of x and y is apparent rather than real, and that the palms of the two boys are in every respect as "identical" as in the other true cases, while that of the girl (z) is quite unlike them. Again the bilateral symmetry of the lefts and rights will be noticed, a peculiarity not seen in the girl (Z).]

(x)	u u u ar ?	? u u u u
(y)	u u u ar ?	? u u u u
(z)	u u u r ?	? u u u u

[There is no reversal of index patterns in the two boys; the left index is in each case a tented arch with a loop on the radial side, the right indices are simple ulnar loops.]

No. XIII.

7. 5. 5. 3. P	9. 7. 5. 3. P
Large θ, trace of Id^1.	Convergence of ridges at hypothenar margin.
9. 9. 5. 3. C	10. 9. 6. 3. C
Large θ, trace of Id^1.	Convergence of ridges at hypothenar margin.

[According to personal appearance these should be duplicates. I have never seen them, but the one who took the prints wrote: "The Misses ——— are so similar in coloring, figure and features that even their best friends confuse them." It must be confessed, however, that the differences in the

formulæ cannot be reconciled, and that the palms are, and remain, in respect to the main lines, very different. They both possess, however, certain peculiar markings in common, as the thenar patterns in the left hands, or the hypothenar convergence in the rights, facts which would help matters out were there any hope of reconciling the lines. I must leave this as a totally aberrant case, and treat it as such in the summary given below. In the other two cases that have caused trouble, Nos. VII and X, the resemblance is not so striking and there are marked differences in height and weight. It will be noted that in these there is a complete lack of that bilateral symmetry in the hands of one individual which is usual, though not invariably the case in undoubted duplicates. Were the theory established beyond doubt, I should unhesitatingly diagnose this as a case of fraternals in whom there happens to be a striking resemblance, but as one cannot be dogmatic, I must leave it as recorded, without explanation.]

u u u u w	w u u u u
u u u u w	w u u u u

[The finger prints correspond exactly in the two individuals, even more than is usual in those twins that are unquestionably duplicates, yet it will be noted that they are in the main ulnar loops, the commonest type of pattern.]

No. XIV.

7. 5. 5. 3. C Indication of $\perp^3$ shown by a broad loop between T^3 and T^4. H a narrow loop.	7. 5. 5. 5. C A broad loop between T^3 and T^4. $\perp^3$.
7. 5. 5. 2. C $\perp^3$ well developed. H a narrow loop, with a core of oblique ridges.	8. 6. 5. 5. P (?) A broad loop between T^3 and T^4 showing rudiment of $\perp^3$.

[These correspond exactly in all but two minor particulars, and show, also, that bilateral symmetry so usual in the case of duplicates. The two differences are slight, and in each case the decision rests upon a single ridge. In the left hand of *x* line A almost runs into the carpal triradius, as it does in the case of *y*, a single ridge in the hollow of the hand deflecting it slightly outward; and in the same way the "8.6" of the right hand of *y* is so near 7.5 that another might interpret it so. These twins I have never seen, but they are reported as being identical.]

u u u u u	u a u u u
u u u a u	u u u u u

[The finger patterns are all ulnar loops except the right index of x and the left index of y, which are tented arches. These phenomena may possibly be of the same order as that of the reversal of patterns in the same finger, since, in passing over from an ulnar to a radial loop, a tented arch is an intermediate stage. Here, at any rate, there is a reversal of hands if not of indices, since the left hand of *y* corresponds to the right of *x*, and vice versa.]

No. XV.

11. 7. 7. 5. S	10. 7. 6. 5. S
A long seam along the hypothenar area and a division of the ridges (=C?) very high up.	Seam, division of ridges, etc., as in *x*-left.
11. 7. 7. 5. S	10. 7. 6. 5. S
Seam, division of ridges, etc., as in *x*-left.	Seam, division of ridges, etc., as in *x*-left.

[This is a very satisfactory instance of true duplicates, since there is not only a complete correspondence between *x* and *y*, hand for hand, but the bilateral correspondence, also, is far greater than the formulæ would indicate. The 10 and 6 of the right hands indicate, of course, that lines D and B meet and fuse, but in the left hands this nearly happens, as in passing they leave between them at the nearest place but two ridges in *x* and four in *y*. In other details they correspond. I have not seen these twins, but their brother states that the resemblance is exact.]

u u u r u	u u u u u
u u u w u	u r u u u

[This finger pattern formula shows the typical nature of this case in another particular, namely, the reversal of the pattern on the right index, an *ulnar* loop on one and a *radial* on the other. What seems to be a lack of correspondence in the left indices disappears when we study the actual prints, since the radial loop of *x* encloses a large oval at its core, which is merely enlarged a little in *y* to form the "whorl."]

No. XVI.

[Used as an example of formulation at the head of this list, *q. v.*]

No. XVII.

9. 8. 5. 5. C	11. 9. 7. 5. C
9. 7. 5. 5. C	9. 7. 5. 5. C

[As these are of different sex they are known *a priori* to belong to the fraternal class. The formulæ are seen not to correspond. It may be noted that *y* is bilaterally symmetrical in the matter of palm patterns, a condition likely to occur, though not very often, in individuals not duplicate twins.]

u u u u u	u u u w u
u u u a u	u u u u u

[No correspondence here.]

CLASSIFICATION OF THE SUBJECTS STUDIED, BASED ON THE ABOVE TABULATION.

An inspection of the above will show that, relying upon the formulæ alone, nine out of sixteen sets, viz., I, II, III, V, IX, XI, XII (boys alone), XIV, and XV, are true duplicates, either absolutely identical

or with one or two slight differences due to the disposal of one or two ridges at some critical point; and that, furthermore, these nine sets are also "identical" in personal appearance. Of the remaining eight, all of which differ as much in the palm and finger formulæ as do brothers and sisters of distinct birth, five of them, viz., Nos. IV, VIII, XVI, XVII, and the girl of No. XII as compared with the boys, are quite unlike in personal appearance; two of them, Nos. VII and X, are very similar but not identical, leaving No. XIII alone to present the irreconcilable data of identical personal appearance with very different formulæ. This set certainly damages the case to a slight extent, but it is but one out of seventeen, or between 5 and 6 per cent, the remaining sixteen being remarkably consistent in their relations to the main theory of the paper. This theory would demand the disposal of No. XIII as a case of fraternal twins in which the two members happen to resemble one another closely, and as such cases may occur in separate births, it is in no way remarkable that in those born at the same time and subjected as far as possible to the same environment after birth a chance resemblance might occur as great as in this instance. The placental condition at birth, employed at the beginning of this paper as an important criterion, would very likely settle the question, but this it is impossible to obtain. As a matter of coincidence it may also be possible to find a case of unlike fraternal twins with very similar palmar formulæ, thus emphasizing the necessity of the identity of both formulæ and good physical characteristics as necessary concomitants in diagnosing a case of genuine identity.

In tabular form the sets studied above may be classified as follows:

True duplicates, with correspondence of physical characters and palmar formulæ, Nos. I, II, III, V, IX, XI, XII (the two boys), XIV, XV..... = 9 sets

Fraternal twins, decidedly unlike both in personal appearance and in palmar formulæ, Nos. IV, VIII, XII (the girl in comparison with the two boys), XVI, XVII (this last of different sex) ..... = 5 sets

Fraternal twins, that look much alike but with different palmar formulæ, Nos. VII and X..... = 2 sets

Twins, probably fraternal, with different palmar formulæ, but strikingly similar in physical characteristics, No. XIII.. = 1 set

Of these the fraternal twins, being in no sense different from children of separate birth, will interest us no further in the present discussion, but the nine sets of duplicates deserve a careful consideration and may now be taken up in detail.

Summary of Friction-skin Conditions in Duplicate Twins.

In the above cases the identity of palmar markings is much more complete than is brought out by the formulæ, since these last have an artificial element in their formation and the course of a line often depends upon the detail of one particular ridge, one of Galton's "minutiæ;" and since the correspondence, even in duplicate twins, is not carried as far as these, a fact that will be brought out later together with the probable reasons for it, it may easily happen that such a slight difference, when occurring at a critical point in the course of a main line, may change the symbol of one or perhaps of two positions. Another source of error is found in affixing a designation to the carpal region, since a print often leaves off at the wrist before the limit of the friction skin is reached, and thus in the case of a very low carpal triradius, this latter may not even appear, causing the investigator to class as a parting what should be a triradius. *In the entire nine sets of true duplicates there is not a single difference that cannot be traced to one of the above sources and thus be shown to be of no real value.*

Features of especial interest in the palmar and finger configuration of duplicates are (1) the tendency to a symmetry between the two sides, which appears to be far greater than among other individuals, and (2) the mysterious reversal of index patterns of one hand or the other. Added to this last there seems also some tendency to lack of correspondence in the thumb patterns, and in a single instance (the rights of No. IX) there is a difference in the patterns of the middle fingers.

A detailed comparison of the nine sets of duplicates considered here yields the following results:

Of these sets five are female, and four (including the two boys from a set of triplets) male. The physical resemblance seems in all cases to be complete, although I can corroborate this from personal inspection in but three of the cases. The total difference in the palmar formulæ, excepting two cases in which the carpal areas are in question, consist of but five instances, all of the left hand, and all of the slight nature explained above. Since each palmar formula plus the character of the carpus consists of 10 symbols, there are in all 90 sets of such symbols in the above list, and if we deduct from these the nine which do not correspond (two of the differences involve two symbols each) we find an exact correspondence of 81 out of the 90 sets or 90 per cent of the whole. To these formulæ may be added, as they occur, 14 instances of other features, such as patterns and lower triradii, 12 of which show a complete and one a partial correspondence, while one alone, the small hypothenar loop in No. V *y*, is unrepresented in its duplicate.

TABLE II.

No.	Degree of physical resemblance.	Sex.	Correspondence of palmar formulæ.	Extent of bilateral symmetry in the palmar formulæ.	Reversal of index pattern.
I.	Identical.*	♀	Complete.	Complete.	Rights.
II.	Identical.*	♀	One difference in left.	Complete with the exception just noted.	Lefts.
III.	Identical.†	♀	Complete.	None.	None.
V.	Identical.†	♂	Two differences in left.	Complete with the exceptions just noted.	Lefts.
IX.	Identical.*	♂	Complete.‡	None.	None.
XI.	Identical.†	♀	Complete.	Complete.	None.
XII.§	Identical.†	♂	One difference in left.	Complete with the exception just noted.	None.
XIV.	Identical.†	♂	One difference in left.‡	Complete with the exception just noted.	Indices show two alternating patterns, *i. e.*, x- left $= y$- right, and y- left $= x$- right.
XV.	Identical.	♀	Complete.	None.	Right.

* Decision rests upon personal inspection.
† Decision rests upon testimony of others, all reliable sources.
‡ In addition a carpal difference not counted because of reasons above stated.
§ The two boys alone from the set of triplets.

A complete bilateral symmetry in the case of duplicate twins calls for a double correspondence, *i. e.*, four hands with the same formula, or, in cases where there is a point of difference, three hands alike and the fourth also corresponding save in the point noted; and yet this difficult requisite is fulfilled in six out of the nine sets, while in the other three there is no attempt at bilateral correspondence. This seems to show that a bilaterality in the case of true duplicates is to be looked for, although it is not a prerequisite.

The occasional reversal of an index pattern is, perhaps, the most singular phenomenon yet observed, but it occurs too often to be a coincidence (4 out of 9, with a strange interrelationship in a fifth). The table shows that this reversal bears no necessary relationship to the bilaterality, since it occurs in No. XV, in which there is no bilateral symmetry, and, on the other hand, is entirely absent in XI and XII, in which a complete bilaterality exists. In its commonest form it consists of the reversal of a loop, which is ulnar in one individual and radial in the other,

but in No. II the reversed pattern is a tented arch to which a small loop is applied laterally, being ulnar and radial respectively in the two individuals. As for the side upon which it occurs, it is right in two cases and left in two. In seeking for an explanation, the theory of the transposition of viscera in twins is recalled to mind, and especially Bateson's statement that the transposition need not necessarily be a complete one, but why the tranposition should affect one finger alone, or why that finger should always be the index, these are at present questions beyond solution.

With the evidence thus far at hand, the impression becomes strong that in typical duplicate twins, the following conditions of the friction-skin configuration ought to co-exist with the correspondence in other physical characters, and probably with that of a single placenta and chorion during development, namely: (1) Duplicate formulæ for the main lines and for the carpus; and (2) Approximate correspondence in other features such as lower triradii, seams, and patterns; to these may be added as usual, though not necessary, (3) Bilateral symmetry between the hands of the two sides, and (4) A reversal of the patterns of one of the indices, either right or left.

DETAIL OF ACTUAL PALM PRINTS OF DUPLICATE TWINS.

For the purpose of enabling the reader to study actual conditions, I have reproduced in Fig. 7, the prints of the left hands of No. I, the tracings of which have been already published (in Amer. Jour. Anat., Vol. I, p. 438). These are good typical duplicates, and although as subjects they may not be as good as those possessing more patterns and other details, they were the only subjects from which I was able to procure prints satisfactory enough to be used for this purpose.

In these the four main lines are identical in their general course and in their effect in the formation of areas, but differ slightly in the relative proportions of the areas they demark. *It is as though identical forces had directed the development in the two individuals, but that the material had yielded a little unequally to the strain of growth, a given area being a little more expanded and consequently covered by a few more ridges in one than in the other.* The result is a slight variation in the curvature of the main lines and in the number of ridges between them at identical spots in the two individuals. Line A, arising from the triradius at the base of the index finger, curves around the base of the

FIG. 7. Prints of the left palms of Twins No. 1. Natural size. The lines of interpretation are marked with india ink. (*Figure on opposite page.*)

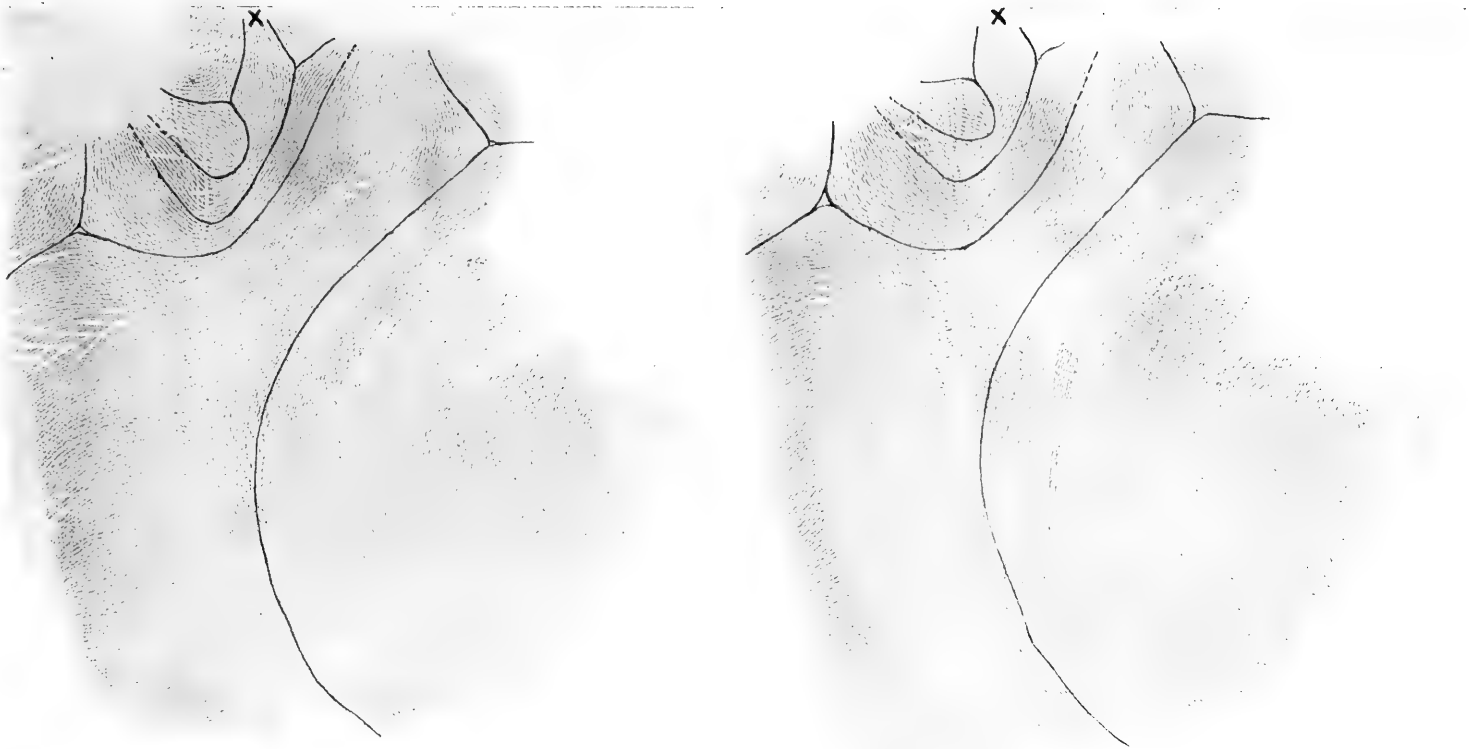

FIG. 7.

thumb and eventually opens at the lower margin on the radial side, an unusual position. The amount of curvature of the line is somewhat less in *y* than in *x*, and the cause of this is evident in the difference of position of the 1st triradius from which it arises, which in *y* is more centrally placed (beneath the middle of the index finger), while in *x* its position is unusually near the inner margin. This difference in curvature is probably also the cause of the difference of relationship between this line and the deep, curved wrinkle, the "line of life," which in *x* is almost coincident with it, while in *y* it diverges widely, especially at its upper end. Lines B and C, those which arise from the bases of the middle and ring fingers respectively, curve outwards and upwards in both *x* and *y* and terminate eventually not far from one another in the interval between the ring and little fingers. Here again slight differences in curvature, and consequently in the shape of the areas which they define, may be noticed, but the similarities are far more striking than the differences. Line D, arising from the base of the little finger, curves around line B in both cases and opens in the interval between the index and middle fingers. The curvature of this line is in *x* slightly more abrupt and as a consequence it comes noticeably nearer to line B along that part of the course at which the two are approximately parallel. In neither of the palms are there especial figures such as patterns or lower triradii.

Farther examples of the correspondence of the main features in duplicate palms may be seen by an inspection of the palm prints of the two boys of the set of triplets (*x* and *y* of Figs. 8 and 9) since these are as genuine a case of duplicate twins as though a fraternal sister had not developed at the same time. These, although smaller and with finer ridges than in the case of adults, are much more convincing studies than are those just considered, since they possess numerous features other than those of the main lines, all of which are duplicated with great fidelity. While studying these, it will be of interest to compare them, point by point, with their fraternal sister, to see the lack of correspondence as well as the similar features which are liable to be found in any children of the same parents, whether contemporary or not.

Here, in the boys, the main lines of both hands are, with one slight exception, as accurately duplicated as in the case of No. I, and the right

FIG. 8. Prints of the left hands of a set of triplets (No. XII), aged four years. Natural size. Lines of interpretation and other features marked with india ink. The two boys (*x* and *y*) are duplicates; the girl *z* is related to the others fraternally.

FIG. 9. Prints of the right hands of No. XII. Natural size. [See explanation under Fig. 8.]

For figures, see opposite page.

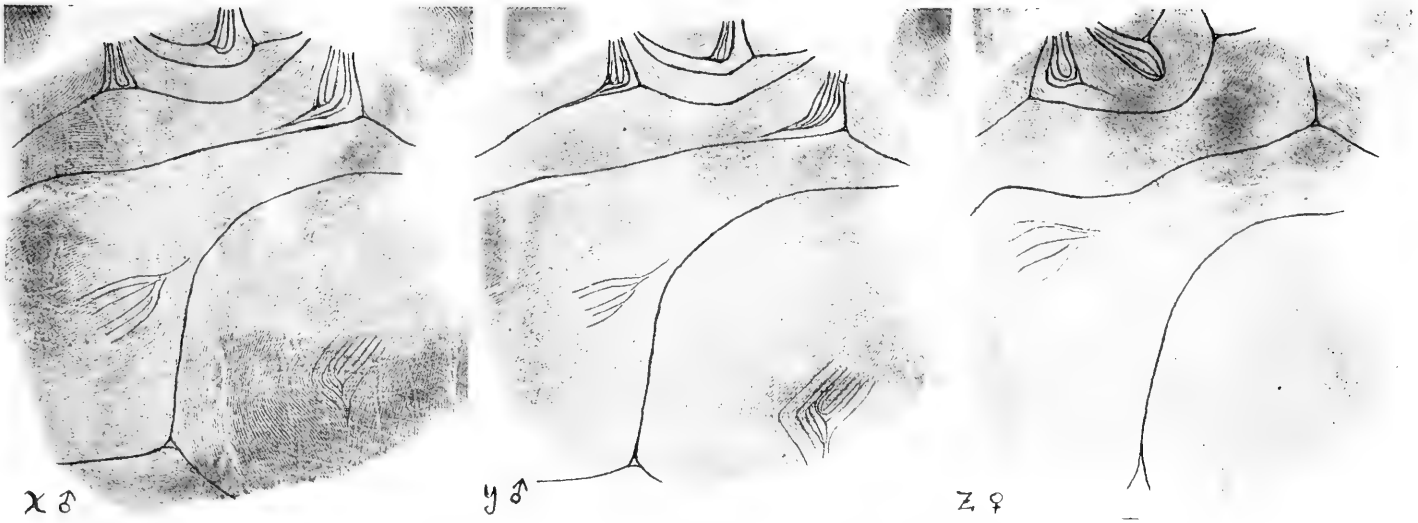

FIG. 8.

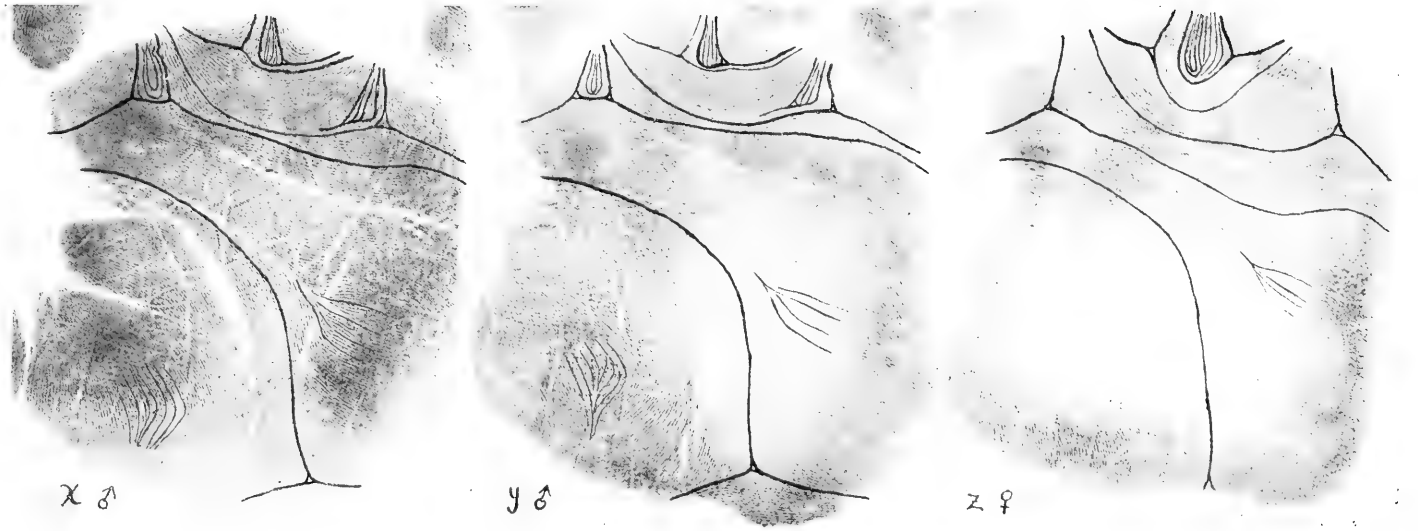

FIG. 9.

and left hands either of the same individual or one of x and one of y, are also exact symmetrical equivalents. The exception just mentioned is hard to find and is so slight that I was for a long time in doubt whether or not to express it in the formulæ as given in the list on page 436. As may be clearly seen by consulting the formula, it is found at the origin of line D in the left hand of y, where by a slight difference in the minutiæ of the ridges, the line arising from the 4th digital triradius cannot be said strictly to become continuous with the 3d lower triradius (ι^3) as in x and in both right hands. (Compare x and y in this particular.) *This case is in itself an interesting contribution to the study of the ridges and their minutiæ, since it shows, as maintained elsewhere, that the exact correspondence of duplicates does not extend to those latter parts save in a general way, and that a difference in main line formulæ often depends upon some eccentricity of a single ridge, in itself sufficient to deflect the course of a line, although of no importance in regard to the general pattern. Six of the eight remaining exceptions to the correspondence of the 90 pairs of symbols embodied in the descriptive formulæ of the nine sets of duplicate twins considered above are of as slight a nature, and the other two differences are situated in the carpal region, and are of still less importance since they are in all probability the result of incompleteness in the prints.*

In the ridges themselves, whether we consider their number or the minutiæ met with along their course, we find but little similarity, although more in the former than in the latter. If, for example, in the two left palms of No. I (Fig. 7) fine straight lines be drawn with a ruler, connecting adjacent digital triradii, and if the ridges crossed by those lines be accurately counted and compared, the results are as follows:

TABLE III.

Area Counted.	No. of Ridges. in x	No. of Ridges. in y
2nd interdigital area (between index and medius).....	49	45
3rd interdigital area (between medius and annularis).....	22	22
4th interdigital area (between annularis and minimus)...	49	51

These differences are certainly not great, especially when we note that there is often a chance for individual difference of opinion, amounting to one or possibly two ridges each way. Thus the pencilled line often crosses an interrupted line at its very end and an almost infinitesimal difference in its position would determine whether or not that line should enter the enumeration. Greater differences than this often

appear if we count the ridges composing areas limited by the main lines, but in these cases we must remember the semi-artificial character of the lines themselves, and the fact that in tracing them there is frequently a point, caused by the forking of a line, the interpolation of a new one or some similar structure, where the decision is an arbitrary one, and that such a variation, although minute at first, may often make considerable difference as the line is continued. For example, we may take in the same prints the two lines B and C at their termination between the 4th and 5th digits. In *x* they are apparently five ridges apart at the margin and but three (or four) in *y*, differences which may easily have been made in two copies of a single hand by swerving the course of the lines as much as permitted by the ridges. There is, however, too much difference in the same two hands at certain other places, as, for example, the distance between lines B and D at the lower curve, to account for it in any such way. In *x* this distance is expressed by an average of seven ridges, while at no place in *y* are there less than fifteen.

This occasional wide discrepancy in the number of ridges suggests that we are on the border between characteristics which are duplicated and those which are not. While the correspondence between the main lines and areas, the patterns and other figures, and even the number of ridges in most cases is nothing short of remarkable, the law seems to fail at about the latter point, and if we turn to the "*minutiæ*" of the ridges, that is, the forkings, interruptions, interpolations and isolations, we find that the limit of resemblance has been passed and that *whatever law of heredity or of construction has caused a similarity of form or arrangement in the larger parts, it is here no longer binding.* Perhaps in this way we may be led to approximate the answer to the question asked by Galton: "What is the minutest biological unit transmissible by heredity?" *since in individuals that arise from one egg and thus possess, presumably, the same inheritance, the main lines, areas, patterns and other large features are duplicated exactly, or as nearly as the ridges will allow them to be, while the ridges themselves with their minutiæ are not.* To illustrate this, apply a lens to the corresponding areas of any two duplicate prints, as, for example, the areas in the left palms of No. I designated by a small x, and follow the details of the ridges. If we compare either the short detached pieces known as "islands," the forkings or the interruptions, we shall see that *in these details the two areas are as individual and distinct as are any two corresponding areas in hands entirely unrelated.* They are like two duplicate pieces of masonry built with irregular blocks of stone taken haphazard, and show clearly that *while in each egg* (*i. e., each*

divided half) *there has been a force or mechanism sufficiently similar to that contained in the other to cause the main lines, the areas and the patterns to develop as practical duplicates, it has attempted no control in the formation of the separate ridges, and that these latter, therefore, have developed in obedience to forces which have appeared later in the development, and which did not exist in the first germ-nucleus.*

Some explanation of these later forces and their method of action has been given through the recent investigations of Miss Whipple, in the work referred to in the bibliography and now in press. The author has studied the genesis of ridges, both phylogenetically and ontogenetically, in the various orders of mammals, and shows that they are formed from either (1) the coalescence of separate *epidermic units,* each with a sweat gland (and, typically, a sebaceous gland and a hair), which arrange themselves in rows and form single ridges, or, in other cases, from (2) *epidermic rings,* formed by a coalescence of the primary units in circles, which, by becoming elliptical and arranging themselves in rows corresponding to their longitudinal axes, form simultaneously two rows of ridges.[3] This process may be easily seen, in all stages of transition, in many mammals, especially Marsupials, Lemurs and the lower Primates, along the borders of ridged areas which are surrounded by either the simple units or by the rings, and in all of these lower forms, in which the friction ridges and the pads are still of functional importance, the separate units *seemingly arrange themselves in obedience to purely mechanical laws,* as though determined through use-inheritance. In fact, I do not know any instance which convinces me so completely of preformation in the egg as the comparison of duplicate twins, nor, on the other hand, one that forms so good an argument in favor of the doctrine of use-inheritance as these investigations of Miss Whipple. The facts and principles thus far brought out in the general study of the surface structures of the mammalian chiridium point more and more to *the great importance of these parts as a basis for the study of fundamental biological problems.*

[3] This development of separate epidermic units into friction ridges, as thus far ascertained, is a phylogenetic development, as traced by the comparison of adult forms. How much of this may be recapitulated in individual ontogeny has not as yet been ascertained, but it seems probable that more is left the individual to accomplish in such lower mammals as Marsupials and Lemurs than in the higher primates. Finger patterns which would probably have become the adult form are demonstrable in a simian embryo of 70-90 days.

Details of Finger Patterns in Duplicate Twins.

It is with much hesitation that I venture upon a field so minutely worked out in every detail by Mr. Galton, the more so as he has already included among his labors a comparison of the patterns of three fingers in the case of seventeen sets of twins,[4] by a coincidence the same number presented here; since, however, he has in this made no distinction between duplicate and fraternal sets, it may not be superfluous to make a short comparison of the formulæ above given, and to present fac-simile prints of an actual case of true duplicates.

My material is in a way incomplete, since I have rolled impressions of but nine of the sets studied (Nos. I, III, VII, VIII, IX, X, XIII, XIV and XV), and for the other cases have to rely upon the dabbed impressions obtained, accidentally, as it were, while taking the general palmar surface. As the fingers are somewhat flattened during this process, the patterns are in most cases sufficiently complete for comparison except in the case of the thumbs of which the edges only appear, save in those few cases in which the operators have had the forethought to make a separate impression of each thumb. Where the pattern of a thumb or other finger is in doubt I have placed a question-mark in the formula. As shown by a cursory examination of the above formulæ, the results in the case of finger patterns are not very definite, and not only is there frequently a lack of correspondence among the duplicates, but there are also cases of undoubted fraternal twins in which the similarity is remarkable. Thus, of the nine duplicate sets, not counting a reversal of indices as a difference, Nos. *II,* III, *V,* XI, and XII (boys) correspond completely, while Nos. *I,* IX, XIV and *XV* show differences (other than reversals), in I the right thumb, in IX the right middle finger, in XIV both sets of indices, and in XV the left index. In all the above the numbers *italicized* show a reversal of an index pattern. Turning now to the seven fraternal sets, No. VII shows four differences, IV and XVI three each, VII, X and XVII, two each, and in XIII alone the two formulæ correspond—the doubtful case. As to the digits in which the differences are located, in one instance it is the thumb, in ten the index, in three the middle finger, and in two the ring finger.

The above results may be more clearly expressed in the form of a table.

Placed in this tabular form the records show at once the much greater correspondence in the duplicate than in the fraternal set, since out of nine sets of the former there are but six differences (aside from reversals), or $6\frac{2}{3}$ per cent, while in the seven sets of the latter there are

[4] Finger-Prints, 1892, pp. 185-187, with Table XXVII.

fifteen, or over 21 per cent of the whole, and in this latter computation there is not included one in set VIII which happens to be a reversal, and is probably a coincidence, since there seems to be in fraternals no especial tendency towards this phenomenon. Again, taking the individual sets and placing over against each the number of differences they exhibit, we have the column at the right hand of the table, which shows very clearly the comparison between the two sorts of twins.

TABLE IV.—SHOWING THE CORRESPONDENCE IN FINGER PATTERNS.

	No. of Set.	Sex.	5	4	3	2	1	1	2	3	4	5	Total Differences.
Duplicates.	I	♀	o	o	o	x	o	x	•	o	o	o	2
	II	♀	o	o	o	•	o	o	o	o	o	o	0
	III	♀	o	o	o	o	o	o	o	o	o	o	0
	V	♂	o	o	o	•	o	o	o	o	o	o	0
	IX	♂	o	o	o	o	o	o	o	x	o	o	1
	XI	♂	o	o	o	o			o	o	o	o	0
	XII (boys)	♂	o	o	o	o			o	o	o	o	0
	XIV	♂	o	o	o	x	o	o	x	o	o	o	2
	XV	♂	o	o	o	x	o	o	•	o	o	o	1
Fraternals.	IV	♀	o	o	x	x	o	o	x	o	o	o	3
	VII	♀	o	o	o	x	o	x	x	o	x	o	4
	VIII	♀	o	o	o	•	o	o	x	o	o	o	1
	X	♀	o	o	o	o			x	x	o	o	2
	XIII	♂♀	o	o	o	o	o	o	o	o	o	o	0
	XVI	♂♀	o	o	o	x	o	o	x	x	o	o	3
	XVII	♂♀	o	o	o	x	o	o	o	o	x	o	2

A correspondence of pattern is indicated by (o), a reversal by (•) and a disparity by (x). Where the material is insufficient to allow a trustworthy comparison, the space is left blank.

No. XIII is a doubtful case. May be duplicate. (See above.)

Again, it must be borne in mind that, exactly as in the case of the palmar features, a difference which would be expressed in a formula is not necessarily of much morphological importance, since here, as Galton shows in spite of his desire to the contrary, patterns do merge into one another by various indefinite steps, and one continually meets with a pattern so near another type that its classification, even by two experts, might well vary.

This can be shown by the finger patterns of No. I, reproduced in Plate B and particularly fitted to illustrate this point, since the twins from which they are printed are unmistakable duplicates, and since the finger patterns show, besides a typical reversal of index patterns, two differences of a degree sufficient to affect the formulæ.

As nearly as I can interpret and apply the Galton system, the formulation of these patterns, in the same arrangement as on the plate, would

be as follows, making use of descriptive suffixes only in the doubtful cases of transition patterns:

TABLE V.

Left.		Right.	
x	y	x	y
u	u	w	u
w	r^w	u	r
u	u	u	u
u	u	w	w
u	u	u	u

Aside from the reversal in the right indices, differences occur in the left indices and the right thumbs. The first of these show in the formula as w and r^w respectively, that is, a whorl and a radial loop forming a transition form towards the whorl type. Galton's distinction between a whorl and a loop rests upon the number of deltas (triradii) present, being two in a whorl and one in a loop. When stated, this distinction seems obvious enough, but since there are all stages in the gradual extinction of a delta (triradius) [see paper by Miss Whipple] it is often impossible to know whether one that is disappearing should be counted or not. Thus, in the case in point, the left index of x certainly possesses two good deltas and is therefore a whorl, but are we to consider that in the corresponding pattern of y one of the deltas (the right in the print) has disappeared or not? It is well indicated by the convergence of the ridges proceeding from the core, and is certainly on the way to extinction, but there is at least room for a difference of opinion concerning its exact status in its present condition. If it is considered as still there, the pattern is a whorl and the two correspond; if absent, the pattern is a loop, and the two differ. Galton himself, usually so clear, is rather unsatisfactory on this point, and refers the reader to four enlarged prints, two of which he calls loops and two whorls, although their differences are extremely slight, and seem almost arbitrary.[6]

The pattern of the left ring finger of x presents the same problem and I have doubtfully referred it to an ulnar loop after considerable hesitation. At all events, it is sufficiently similar to the corresponding pattern of y to be considered a duplicate, and it would be manifestly misleading to place it in the formula as a w, and thus introduce what would count as a difference.

[6] Finger-print Directories, 1895, p. 109, and Plate 8, bottom line.

The other difference, that of the right thumbs, presents a somewhat greater difficulty, but morphologically the patterns are very similar and in order to convert the pattern in x into that in y all that is needed is to complete the breaking down of the weaker triradius (the right in the print), which has already begun, leaving all other ridges exactly as at present.

The reversal of index patterns as seen in the right hands is a good typical example of that frequently recurring phenomenon, and seems to occur with too great frequency (four cases out of nine) to be disposed of simply as a lack of correspondence like the rest. The other cases, as has been shown, are usually or always those of transition patterns not really unlike morphologically, but in this case one pattern is the exact symmetrical equivalent of the other, and hardly capable of becoming identical by anything less than a complete rearrangement of the entire pattern. These reversed patterns may be better studied in the enlarged figures of their cores, represented in (a) of Fig. 10.

As in the case of the palms there is little or no attempt at correspondence in the minutiæ, as may well be seen by applying a reading glass to the cores of the patterns in Plate B, or by a study of the enlarged cores of Fig. 10, which show those of the indices, middle and ring fingers of the two right hands. The various ulnar loops, for example, show several types of Galton's "Secondary Classification" without any correspondence between the two individuals; thus the single rod expressed by the descriptive suffix "i," appears in x-left, middle finger, in y-left, thumb and middle fingers, and in x-right, middle and little fingers. The eyed rod, suffix "f," appears in x, right index, and in conjunction with a single rod in x, left thumb. The staple, suffix "c," appears in x-left, little finger, in y-left, little and ring fingers, in x-left, middle and little fingers, and in y-right, little finger. In all this there is some little correspondence, as in Fig. 10 b, but that it is probably a coincidence appears from the lack of correspondence in the other minutiæ, as is easily seen by a more complete scrutiny of the figure just referred to.

FIG. 10. Cores of finger patterns from set No. I. Compare with Plate B.
(a) Right indices. *Note the Reversal.*
(b) Right middle fingers.
(c) Right ring fingers.

In close connection with the present subject come investigations of

the finger patterns of twinned fingers in those cases of hyperdactylism in which it is probable that the extra digit may be referred to a doubling of one of the normal ones, as in a double thumb. I have received prints of two individuals exhibiting this phenomenon, in both cases on the right hand alone, and have special thumb prints of one of these. I had naturally expected, *a priori,* that the patterns of the two right thumbs would prove to be duplicates of one another, but such is by no means the case. In this individual the "outer" (external or radial) thumb shows a typical radial loop, a rare pattern for a thumb, while the "inner" (internal) thumb is marked by a simple arch. The left thumb presents an ulnar loop. That the supernumerary digit is rightly classed as a thumb is shown by its origin, evidently from a common 1st metacarpal, and the two thumbs are united as far as the middle of the proximal phalangeal joint, and lie so near one another that the rolled prints were taken with considerable difficulty. The pattern of the right index finger adjacent to the inner thumb is an ulnar loop. Of the other individual with double thumbs I possess only the general print of the volar surface of the hand, but in this the inner thumb is turned in such a way as to suggest that its pattern, as in the other case, is a simple arch. This conclusion is not absolutely reliable on account of the incompleteness of the print. In addition to the above, five cases of hyperdactylism in which the supernumerary digit is a post-minimus, have come under my observation, but as in all of these the extra finger had been removed the data obtained were those of the palm alone.

It would be premature to offer any conclusion based upon a single observation, but it may be allowable to point out that the occurrence of patterns of distinct types upon the two terminal phalanges of a "double-thumb" contrary to all expectation, is naturally in opposition to all theories that suggest a splitting of the anlage, a double set of determinants or any cause involving a duplication of parts, as an explanation of such a phenomenon. If, with Zander, we believe that an originally single anlage is split by the tension of amniotic threads, or if, with Tornier, we consider one of the thumbs the result of super-regeneration from the other, or "Stamm-individuum," we must in some way account for the total lack of resemblance between the two resulting parts. This would seem a fruitful field for investigation, and the comparison of the finger patterns of supernumerary digits may lead to interesting results.

Summing up the results obtained from a comparison of the finger-patterns of the two types of twins, they corroborate in general the conclusions reached from a similar comparison of the palms, although it seems as though the correspondences are not of as exact a nature as is

the palmar configuration, a fact which may be due to the greater importance of the individual ridges in the formation of the patterns, and to the close morphological interrelation between the four primary types. It may be definitely stated, however, in the case of duplicate twins (1) that the finger patterns correspond far more completely than in those of the fraternal type, (2) that the differences are more apt to appear in the thumbs and indices, and (3) that these latter patterns show a strong tendency in one hand or the other towards a reversal, *i. e.*, the formation in the two individuals of patterns which are the symmetrical equivalents of one another. To these conclusions may be added (4) that twinned digits in a single individual, at least in the case of double thumbs, do not necessarily possess duplicate patterns.

The Sole Configurations in Twins.

As material for this study I have the sole prints of seven sets, five being duplicates (I, II, III, XII, XV) and two fraternal (IV, VII). As far as it is safe to draw conclusions from so small a number *they exhibit the same principles as those shown by the palms and are in some ways rather better for study since they often possess a more complex configuration. In general, it may be said that the soles of duplicates exhibit the same striking correspondence in main lines and in patterns as do the palms, as well as the same tendency to a bilateral symmetry when the two sides are compared; also that in fraternal twins there is either a striking contrast or else the similarity, at best, is no greater than among other members of the same family.*

In detail the observations of the different sets are as follows:

Duplicates.

I. In these the four soles are all exact duplicates and belong to what may be called the simple type, one in which the four main lines and all intervening ridges cross the inner margin and form no loops or patterns during their course. The only pattern present is the 1st interdigital or hallucal (the "thenar" of previous articles) which is reduced to a simple loop opening upward between the 1st and 2nd toes. There is no definite lower triradius on any of the feet, but there are rudiments of one in all, situated between lines B and C.

II. In this set the right footprint of *x* is wanting owing to a slight injury received just previous to the printing and rendering it unwise to attempt a print. The comparison is thus confined to the two lefts, but as the remaining right, that of *y*, is the symmetrical equivalent of the others, there is little room to doubt that the missing foot would also be a duplicate of the other three. The configuration of these soles is rather complicated and as it shows with especial clearness the principle expressed in the case of the hands, *that*

of the formative force determining the areas but allowing their relative extent to be determined by the stress of growth at the different points, the two left soles have been reproduced here, covered by their lines of interpretation (Fig. 11). In these it will be noted that of the main lines, D fuses with the lower triradius; C curves inwards and opens between digits III and IV (or their corresponding triradii); B curves outward, embracing C and opening between the triradii of digits IV and V; while A differs slightly in the two individuals, fusing with the lower triradius in x and missing it by three ridges in y. This is the only difference in the main pattern in the two soles and may easily be attributed to a slight difference in the ridges, since their courses lie at one point so near one another. The hallucal line at the base

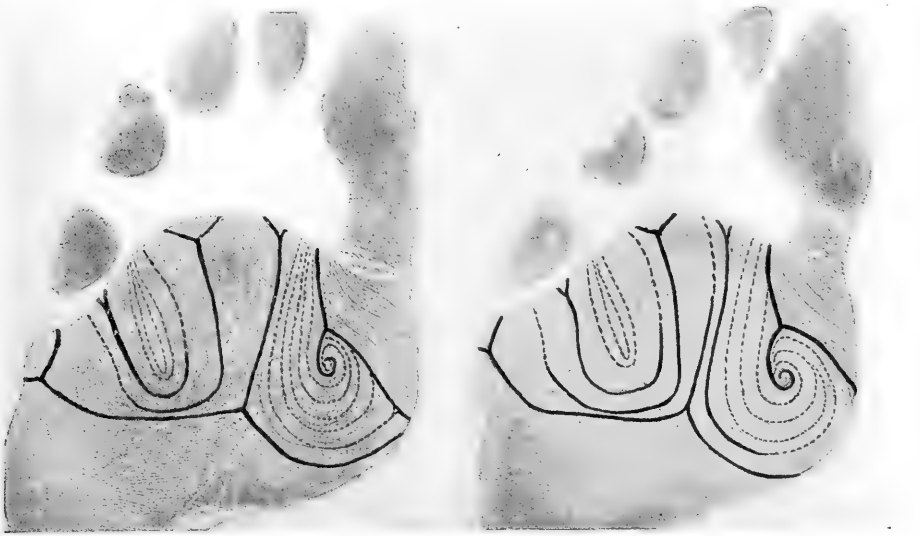

FIG. 11. Prints of the left soles of twins No. II. Natural size. The lines of interpretation are marked with india ink.

of the hallux forms a spiral core for the hallucal, or 1st interdigital pattern, the general configuration of which is marked by the dotted lines. Both in this case and in that of the other pattern, the 3rd interdigital, the ridges picked out by the dotted lines are taken arbitrarily, as the only purpose is to show the general trend of the ridges in the region thus marked, and thus no special significance should be attached to their total number in either case. The five dotted lines defining the hallucal pattern of x, for example, as compared with the three (or four) in y mean nothing more than, perhaps, a slightly greater width to be defined in the latter case, and the two that happen to be used in each case to define the loop in the 3rd interdigital area are intended to point out the identity in shape in the two patterns, with no reference to the number of ridges involved.

III. Tracings of this entire set have been already published in the American Journal of Anatomy, Vol. I, p. 437, and exhibit again the same principles as

do the previous ones, duplicity of the general patterns in the two individuals, and a bilateral symmetry of the two sides. As in No. II, the configuration will be found to be almost but not entirely identical, the only difference in the left feet being due to a slight shifting of a lower triradius which deflects line A in the one case to the outer and in the other to the inner margin. in the right foot a digital line, the 4th, becomes recurved and lost in a pattern in one individual and not in the other. The remainder of the sole is the same in both.

XII (the boys of the triplet set). The feet are typical duplicates with symmetry between the right and left. The commonest characteristics of all four are the following: (a) hallucal patterns of the simple loop type, opening upward between the hallux and the second toe; (b) broad loops circumscribing the 3rd interdigital area; (c) a large lower triradius between the hallucal and 2nd interdigital patterns, with radiants running to the inner and outer margin, and upward. The course of these latter varies a little, making slight differences like those shown in Fig. 11; (d) a curve around the upper border of the 2nd interdigital area; this curve is not quite complete in one case. Aside from the variation mentioned in connection with the lower triradius, the only other one possible is in connection with the appearance of a hypothenar loop in one case (Y-left). The prints, however, do not prove that this is not present in the other cases as well, since they are not rolled, and this pattern is usually beyond the limit of an unrolled print.

A comparison of the girl with her brothers is interesting, and shows the same decided lack of correspondence as in the palms (Figs. 8 and 9). The soles are of the smooth, featureless type, and consist of oblique, approximately parallel ridges without loops, curves or lower triradii. A rudiment of one of the latter may be made out in the right foot. In the left foot there is a pronounced calcar loop on the inner edge of the heel.

XV. The sole patterns are typical duplicates with no essential variations in the curve of the main lines nor in the types of the hallucal patterns. In the left feet there is a large and conspicuous lower triradius, whose radiants extend outwards, upwards and inwards, across the entire ball. In both, the upper or ascending radiant pushes up between the hallucal pattern and the outer opens at the margin just below the origin of line D. Lines B and C curve around a large 3rd interdigital loop, passing each other in the same relation in each case. Lines A and D meet and fuse. The hallucal patterns are spirals, formed by the hallucal line and directed toward the inner margin. The configuration of the right feet is almost a duplicate of the above, and the two are exactly in accord with one another. In these line D fuses, not with line A, but with the outer radiant of the lower triradius, and line A passes within the loop thus formed. The 3rd digital line in both becomes recurved, forming a loop about the upper end of the 2nd digital area. In one point alone the two right soles show a difference, and that is in the presence in x of an extra upper triradius between digits II and III, but as it appears at the very margin of the print, and is barely included at that, it is more than probable that a similar triradius is borne by y a little higher up and just beyond the limit of the print. The hallucal patterns are spirals formed by the hallucal lines and are the symmetrical equivalents of those in the left feet.

FRATERNALS.

VII. These prints are fairly similar, as, it may be remembered, their personal appearance is also, all of which may be correlated as a common inheritance in which they both have shared, inheriting similar qualities, or a similar combination, affecting all parts of the body. In this connection it would be interesting to note how great may be the tendency towards similarity in palms and soles between two children of different births who closely resemble one another in appearance.

In detail, the left feet are alike in the hallucal patterns, which form in each case a simple loop; and in the general course of the main lines. There is, however, in *y* an important 2nd lower triradius, the two upper radiants of which form a broad looped pattern on the 3rd interdigital area, all of which is absent in *x*, or suggested merely by a convergence of ridges in the position of the missing triradius. In *x* the 2nd digital line curves downward, enclosing the 2nd interdigital area, while in Y this line is normal and the 3rd digital line curves upward.

In the right feet the hallucal patterns are as in the left, but the relations of the first three main lines become very different through the presence of a 1st lower triradius in *y*, wholly absent in *x*. Both have a looped pattern upon the 3rd interdigital area, but the relation to it of the main lines is quite different in the two.

As these details are hard to follow without diagrams, they may be summarized in the statement that the soles are quite similar to one another in general appearance, but possess much greater difference than has yet been observed in either palms or soles of genuine duplicate twins.

IV. These prints are totally different in every respect; in the general shape the feet of *x* are long and narrow, while those of *y* are shorter by 1.4 cm. and much broader. I have never seen the individuals from whom the prints were taken, but am informed that they bear little or no resemblance to each other.

The sole configuration is of much interest, being as different in the two individuals as was to be expected from the other data. This is seen most strikingly by a comparison of the hallucal patterns. In *x* the patterns on the two feet correspond, presenting the rather uncommon type of a loop that opens upon the inner margin, enclosed by the incurved hallucal line; in *y* the two are very different from one another and from those of *x*, that on the left foot being a spiral with two triradii and that on the right a simple loop that opens upward between the first two digits. The remainder of the foot in *y* is perfectly featureless, its ridges crossing the base of the foot obliquely, and with a slight convergence towards the inner margin; in each foot of *y* there is a conspicuous rounded loop over the upper end of the 3rd interdigital arch in which the 2nd digital line participates, and in the right foot there is a hypothenar loop.

By this comparison it is seen that the complete lack of correspondence in the soles is in perfect accord with the other data, the palms, the finger patterns, and the personal appearance.

Physical Measurements of Duplicate Twins.

The physical measurements are far less determinative than are the other characters employed since they are liable to fluctuations through numerous causes, both external and internal, and it could hardly be expected that the similarities here would be very striking.

There is often, however, much correspondence in the events of life since, while children at least, duplicate twins are usually closer companions than in the case of most children. Such twins almost universally use the pronoun "we" when referring to themselves,[7] or when relating past experiences, and one young lady, a duplicate twin, confessed that she never felt like kissing her twin sister, on the ground that the latter did not seem like a distinct person. Cases are common in which duplicate twins are affected by the same diseases at the same time and with about the same result, and I know of two little twins with straight hair, who experienced typhoid fever together, after which their hair came out curly in each case. This constant and close companionship and participation in mutual experiences would naturally tend to a uniform development in each up to the period of adult life; but from this point on, in the majority of instances, the twins separate and the varied experiences to which they then become subjected usually produce more or less marked differences in their later physical development.

Physical statistics, then, in order to be of value, should be taken during the younger life, or at least before there is any marked difference in experience, and in the four cases here presented (Table VI) these conditions are met with, as they are all those of young people, the age of each at the time of measurement being designated in the table. The Roman numerals are those of the sets in my collection as used elsewhere in this paper. The capital B, placed at the extreme left, designates a measurement as one that is employed in the Bertillon system. The measurements are in millimeters. In the next to the last line, under the item *Weight,* I, II and III are given in pounds, VI in kilos.

These statistics show that in twins of the age here represented there is quite a little difference, both in girths and lengths, although, as may be expected, there is a greater difference in those which depend upon

[7] This form of language, almost as distinctive, when used habitually, as the "thee" dialect of the Quakers, is well shown by the following extract from a letter from one of the sets in reply to a request for prints: "Our brother told us that we might hear from you and we were interested in your articles and shall be glad to take some prints of ourselves."

TABLE VI.—MEASUREMENTS OF FOUR SETS OF DUPLICATE TWINS.

	Designation of Measurements.	I		II		III		IV	
	Age when measured.....	21.1	21.1	17.11	17.11	17.10	17.10	17.11	17.11
B	Height	1668	1682	1671	1656	1632	1631	1734	1740
	Horizontal length.....	..	..	..	..	..	..	1760	1760
	Height, sternum.....	..	..	..	..	..	..	1409	1422
	" navel	..	..	..	..	..	..	1033	1030
	" pubes.....	..	..	..	..	..	..	877	884
B	" sitting	..	..	..	..	..	..	890	900
	" knee	..	..	..	..	..	..	494	490
	Girth, head.....	..	..	..	..	..	..	570	565
	" neck.....	..	..	..	..	..	..	339	350
	" chest, repose.....	750	758	700	720	745	752	825	890
	" chest, full.....	820	826	752	762	807	820	870	930
	" 9th rib.....	720	705	600	635	620	630	..	..
	" 9th rib, full.....	758	750	660	685	695	703	..	..
	" waist.....	624	582	590	598	560	550	712	730
	" hips	932	952	865	905	915	930	852	870
	" right thigh.....	512	466	475	510	495	520	491	510
	" left thigh.....	510	461	470	512	495	502	490	500
	" right knee.....	..	..	..	..	..	..	340	352
	" left knee.....	..	..	..	..	..	..	350	345
	" right calf.....	323	331	330	338	335	322	342	350
	" left calf.....	320	327	335	340	310	317	356	355
	" right instep.....	..	..	..	..	..	..	245	245
	" left instep.....	..	..	..	..	..	..	249	250
	" right upper arm..	250	246	225	240	245	244	240	240
	" left upper arm....	245	243	230	240	234	243	232	235
	" right elbow.....	..	..	..	..	..	..	250	230
	" left elbow.....	..	..	..	..	..	..	240	227
	" right forearm....	243	244	225	227	219	222	255	255
	" left forearm.....	239	238	232	227	246	215	240	240
	" right wrist.....	..	..	..	..	..	..	162	165
	" left wrist.....	..	..	..	..	..	..	160	160
	Depth, chest	172	170	138	150	171	162	..	..
	" abdomen.....	165	178	122	122	123	120	..	..
B	Breadth, head.....	..	..	..	..	..	..	150	154
	" neck	..	..	..	..	..	..	99	111
	" shoulders.....	374	367	357	362	329	344	595	425
	" nipples.....	..	..	..	..	..	..	185	185
	" waist.....	190	197	191.9	189	162	164	247	254
	" hips.....	307	316	322	328	268	278	308	322
	Length, right shoulder elbow.....	..	..	..	..	..	..	377	366
	" left shoulder elbow.	..	..	..	..	..	..	364	356
	" right elbow tip....	..	..	..	.	..	..	462	464
B	" left elbow tip.....	..	..	..	..	..	..	463	454
	" right foot.....	..	..	..	..	..	..	262	255
B	" left foot.....	..	..	..	..	..	..	260	256
B	Stretch of arms.....	..	..	..	..	.	..	1784	1770
	Strength, back.....	60	60	65	75	105	101	154	170
	" chest.....	28	22	28	34.2	28.2	26	..	..
	" legs.....	70	70	105	102	115	105	175	175
	" right forearm....	27	23	26	25	26	26	38	42
	" left forearm.....	23	18	21	19	21	22	36	32
	Capacity of lungs.....	1.50	1.45	1.40	1.60	1.55	1.63	3.85	4.55
	Total strength	..	..	..	..	..	..	404	479
	Weight.....	115	117.5	107	113	113	114.5	57.1	59.3
	Pilosity	..	..	..	..	..	..	2.2	2.1

the soft parts than in those dependent upon the skeletal parts. Even here, however, there is some difference, as in the standing height, which differs respectively by 14, 15, 1 and 6 mm. The difference in breadth of head in No. VI is of interest, since the skull would be but little affected by environment, and it would be a matter of great interest if we could have the breadth in the other cases and the length in all, that a comparison might be made of the cephalic indices. The marked difference in chest girth in No. VI would seem to point to a differing degree of interest in athletic sports possessed by these young men, but the difference in this item appears to be considerable in the other cases also, and is correlated with a variation in the breadth of shoulders save in I, in which the difference is on the wrong side. Unexpected differences are found in such items as the length of feet and the length of cubitus (left and right elbow tips) in No. VI, since they are based on skeletal parts. The variations in girth are more to be expected, as they are easily influenced by varying causes, such as amount of exercise, condition of the digestive organs, etc.

SUMMARY.

As this paper has drawn so largely on previous work and, in the exposition of its theories, makes use of so many facts and principles that are already well known, an attempt will be made here to separate these from the immediate results of the present investigation, and to that end I will divide this summary into two parts, in the first of which, headed "Results," I will state the essential points of my own investigations, and will follow this by the "Conclusions," in which I will attempt a condensed statement of the theories held by this paper, without reference to the sources from which the material has been derived. It should be emphasized that the "Conclusions" cited here are not stated as facts already proven, but as the various parts of a working hypothesis seemingly consistent with the facts so far as known at present, and intended to suggest farther speculations in this field.

I. RESULTS.

1. My material has been derived from sixteen sets of twins and two sets of triplets, and includes:

a. Complete palm and sole prints of one set of triplets.

b. Complete palm and sole prints of six sets of twins.

c. Palm prints of nine other sets of twins.

d. Rolled finger prints of seven of the sets of twins; dabbed finger prints of the remainder.

e. Physical measurements of three of the above sets of twins and of one set of which I have no prints.

f. A photograph of a set of triplets.

2. The prints allow themselves to be classified in two distinct groups:

a. Those in which the main lines and other features of palms, soles and finger prints correspond to a remarkable degree.

b. Those in which the features just named show no greater similarity than in any two brothers and sisters of distinct birth, or between individuals not related.

3. These correspondences (those in the first group, 2 a) are limited to the course of the main lines of interpretation, and to the type, position, etc., of patterns or other macroscopic peculiarities, allowing some latitude in the relative size of the various areas. There is no correspondence in the characters of the individual ridges (the minutiæ of Galton).

4. In the case of the finger patterns the correspondences are subject to the following exceptions:

a. The patterns of the index fingers are frequently different from one another, a condition occasionally met with in the thumbs, and once (in the present investigation) in the middle fingers.

b. In almost 50 per cent of the cases of general correspondence examined (4 out of 9), the patterns of one of the indices, either right or left, are of the same type in the two individuals of the set, but are exactly reversed, being the symmetrical equivalents of each other.

5. In the case of the palm and sole configuration, where there is a correspondence of main lines and other features, there is also usually, though not always, an approximate or exact correspondence between the markings of the right and left side, a relation which occurs occasionally in an individual not of multiple birth, but infrequently.

6. This correspondence in the configuration of the friction-skin of hand and feet is in all cases (with the exception of No. XIII) correlated with that marked correspondence in the physical appearance, including the facial features, which constitutes the type of twin commonly known as "identical," here called "duplicate"; where, on the other hand, there is a lack of correspondence in one of these details, there is in the others also. These latter are called here "fraternal" twins. Out of sixteen sets of twins examined, nine sets of duplicate and six sets of fraternals exhibited the above principles in detail, and of a set of triplets, which consists of two boys and a girl, the boys were typical duplicates, while the girl, as related to the boys, was fraternal.

7. In set No. XIII, although the two individuals closely resemble one

another, the details of the palms and soles do not wholly correspond. There seems at present no very satisfactory way of explaining these, unless it be to consider them (1) as true duplicates, and allow the possibility of some lack of correspondence in the friction-skin configuration, or (2) as fraternals that chance to look very much alike. Of these alternatives the second is here considered the more probable.

8. Physical measurements of four sets of duplicate twins between 17 and 21 years of age show that they are not identical in size, although the variations are for the most part slight. Differences that depend upon skeletal parts are less than those depending upon the soft parts.

II. CONCLUSIONS.

A. On Twins and Their Relationship to Double Monsters.

1. Twins belong to two types, *duplicate* and *fraternal.*

2. *Fraternal twins* result from the simultaneous ripening and consequent fertilization of two separate eggs, and are thus as distinctly different as are any other two children of different birth. They may be of the same or of different sex; each develops within a separate chorion and possesses a separate placenta; they may or may not resemble one another; the palm, sole and finger markings do not correspond.

3. *Duplicate twins* are the result of the total separation of the first two blastomeres of a single egg, the product of the first cleavage, and therefore possess an identical germ plasm. They are invariably of the same sex; they develop within a common chorion, but possess each a separate umbilical cord attached to a common placenta; they greatly resemble each other, usually to the point of confusion; the palm, sole and finger markings correspond in detail as far as but not including the minutiæ.

4. *Symmetrical double monsters* (*diplopagi*) are closely related to the last, and result from a partial, instead of a total separation, of the first two blastomeres, the separation being sufficient to cause a loss of continuity and hence of relation, over a greater or less extent of surface. The components of such monstrous births are the physical duplicates of one another, and will doubtless be found to correspond in regard to palm, sole and finger configuration, as do separate duplicate twins. The double monsters of which we have authentic record are sufficiently numerous and diverse to represent every stage from that of an otherwise normal individual with a doubling of certain of the median parts to that of two complete duplicate twins with a slight connection between them. They may also be arranged to represent several developmental

series differing geometrically from one another and corresponding to variations in the place of separation of the first two blastomeres, or in their relative position.

5. *Unequal double monsters* (*autosite* and *parasite*) are the result of a secondary fusion of two embryos, owing to a too great contiguity. It is probable that these are at first duplicate twins, the enclosure of which within a common chorion would furnish the crowded conditions necessary for such a fusion.

B. On the Architecture (Promorphology) of the Ovum.

1. The normal mammalian egg, at least from the beginning of cleavage, possesses a definite architectural plan, having a fixed relation to that of the adult body. It seems probable that this plan is bilateral, and that, as in the frog's egg, the first two blastomeres are right and left, and become the progenitors of the two sides of the adult, except that here they must give rise also to the two halves of the chorion and other extra embryonal parts.

2. A change of relationship in the early blastomeres will modify the development of each, as has been shown experimentally in the case of lower animals. In changes affecting the first two blastomeres, we may draw the following conclusions:

a. When they remain together in the normal position, the mutual contact upon the inner sides causes each to develop as a bilateral half.

b. When they separate and the inner sides, once in contact with each other, become external, *i. e.*, placed in the same relations as are the other sides, each will develop the other half body as in the case of the entire ovum, and produce duplicate twins. This shows that the power to develop an entire organism when placed in the proper relations is retained by each of the early blastomeres up to a certain point, as has been proven experimentally to be the case in numerous lower animals.

c. When a separation of the first two blastomeres is incompletely effected, the parts that lose the contact relations develop independently as parts of entire individuals, while the parts that remain in normal contact develop each a half as usual. The various possibilities of partial separation give rise to the various types of symmetrical double monsters (*diplopagi*).

C. On the Composition of the Germ-Plasm, and the Size-Limit of Hereditary Control.

1. It is undeniable that the facts presented in this paper point to some form of preformation, that is, to a mechanism in the nucleus of the

fertilized ovum which controls the development down to considerable detail. If the premises concerning the genesis of duplicate twins are true, we have given us by nature the convincing experiment of the separate development of two identical eggs, containing identical bits of germplasm, derived from the same original male and female germ-nuclei.

Indeed, we have for comparison the control experiment of the development of two different eggs simultaneously in the same uterus and under identical conditions, with results as different as might be expected from the premises, showing that the correspondence in the one case and the lack of it in the other are both based upon the composition of the germnuclei and are in no way affected by subsequent conditions of development.

2. Concerning the correspondence of palm and sole patterns in the various sorts of twins (and triplets) the present investigations have yielded the following results:

a. Out of ten sets in which the physical resemblance was sufficiently remarkable for them to be considered duplicates, nine corresponded so completely in the palm patterns that out of 104 points compared (main lines, triradii and patterns) 94 were exact duplicates, and of the ten differences, two were probably due to an incompleteness in the print, while the remainder were very slight and usually due to an aberrancy in a single ridge. The tenth set (No. XIII) did not correspond.

b. In seven sets of twins that did not resemble one another (including the girl triplets as compared with the boys), there was no more correspondence between the palms of the two individuals than is usual in members of the same family.

c. The study of the sole prints of seven of the duplicate and two of the fraternal sets (all that I have) yielded the same results as in the case of the palms.

d. The finger patterns of duplicate twins are very similar, but in place of exact correspondences there sometimes occur patterns which are easily derived from one another but which would have different formulæ in Galton's system. The differences of this kind are especially apt to happen in the index fingers; they sometimes occur also in the thumbs, and have been noted once in middle fingers.

e. In the case of duplicates the following additional phenomena have been observed in the majority of cases, but are not universal:

(1) A bilateral correspondence in the two palms or soles of each individual of a set.

(2) A reversal of the finger patterns in either the right or the left indices.

(3) Differences occur much more frequently on the left side.

3. The influence of the germ-plasm and its mechanism (*i. e.,* the direct control exercised by heredity) is exerted upon the friction-skin surfaces only so far as concerns the general configuration, *i. e.,* the main lines, the patterns and other similar features; the individual ridges and their details (*minutiæ*) are apparently under the control of individual mechanical laws to which they are subjected during growth. *Have we then arrived at the limit of the control of the predetermining mechanism beyond which mechanical laws are alone operative; and is it then possible to hold that the modifications in this latter field are the results of individual experience, and that they are similar in the various members of a given species solely because of similar environment?* To these and similar questions we can have no answer at present; yet it seems likely that in the general subject of palm and sole markings, not only in man but in other mammals as well, we have a set of easily observed and very significant data which may yield important results to future investigators.

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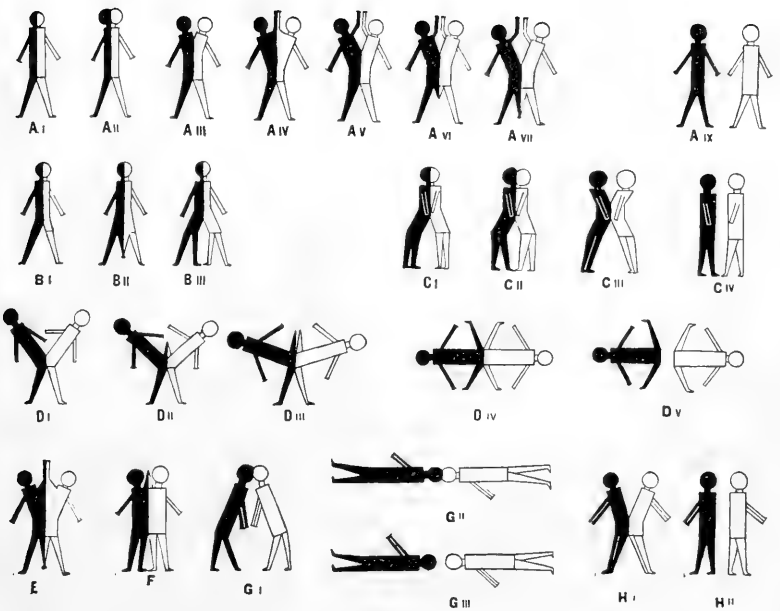
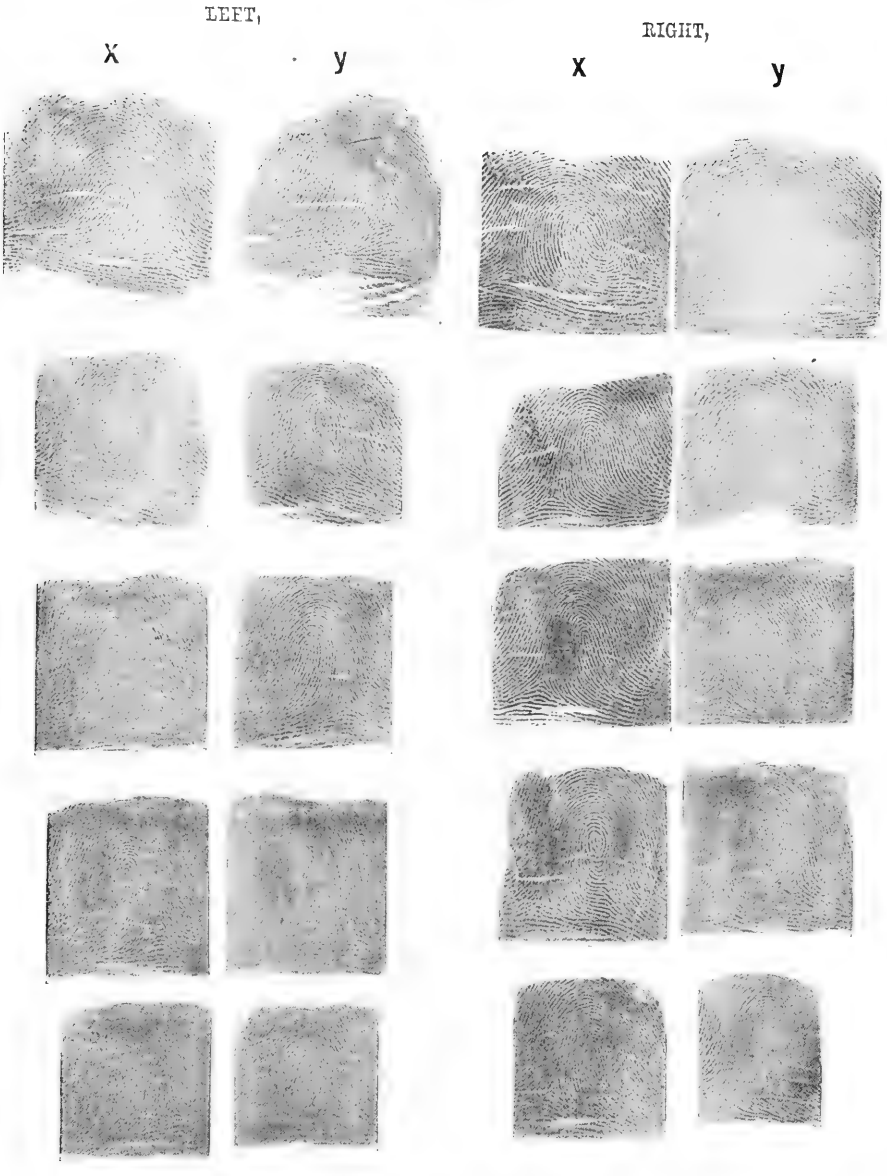


DIAGRAM SHOWING THE INTERRELATIONS OF THE VARIOUS SORTS OF DIPLOPAGI AND DUPLICATE TWINS, ILLUSTRATIVE OF THE THEORY ADVANCED IN THIS PAPER. FURTHER EXPLANATION IN THE TEXT.



FINGER PATTERNS OF TWINS NO. 1. THE TWO PERPENDICULAR ROWS TO THE LEFT REPRESENT THOSE OF THE TWO LEFT HANDS; THE TWO ROWS TO THE RIGHT, THE TWO RIGHT HANDS. IN EACH CASE THE LEFT HAND ROW IS THAT OF INDIVIDUAL X, THE OTHER THAT OF INDIVIDUAL Y.

A CONTRIBUTION TO THE EMBRYOLOGY OF HYLODES MARTINICENSIS.

BY

LILIAN V. SAMPSON.

WITH 2 PLATES AND 17 FIGURES IN THE TEXT.

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For many years the general outline of the embryonic history of Hylodes Martinicensis has been known. Since the early seventies, it has been described as the West Indian tree-frog, with no aquatic tadpole stages, but with an embryo developing within the egg membranes as far as the adult form,—the embryo being provided with a large supply of yolk food, being devoid of gills, but with a highly vascular and, therefore, it was believed, respiratory tail. I am fortunate in having at my disposal a good series of the eggs of Hylodes, from which I have studied the development in more detail.

I am glad to take this opportunity of acknowledging my indebtedness to Professor T. H. Morgan for the material which he has collected and obtained from others at different times, and for his help and suggestions in the work.

The eggs that I shall describe were collected by Professor Morgan and Dr. Conant during two or three seasons, in the month of July, on expeditions of the Marine Laboratory of the Johns Hopkins Univer-

sity to Jamaica. The eggs were found at Bog Walk, on Blue Mountain Peak, and at Port Antonio. On Blue Mountain Peak they occur at a high elevation (about 6000 feet) in very damp localities, where the rainfall is very heavy.

Andrews, **92**, reports that "these tree-frogs are found all over the island; at Port Antonio the breeding season seemed to be past July 20; while at Manchioneal, specimens with very large ovarian eggs were taken July 24."

I wish also to thank Mr. E. L. Griffin for a set of eggs collected at Cinchona (an elevation of 5100 feet).

The eggs are found imbedded in a clear gelatinous substance, in masses of about an inch in diameter, under loose stones or logs. During their development, the adult frogs are to be found near them. It appears, from the preserved material, that, in young stages at least, all the eggs of a mass are uniformly advanced in development.

I. EXTERNAL CHARACTERS.

1. SEGMENTATION.

The segmentation stages that were obtained are sufficient to show the general character of the division of the egg. In spite of the large quantity of yolk, the segmentation is holoblastic. The first furrow usually divides the egg in half, and the second comes in at right angles to the first (Plate I, Fig. 1[1]), but in one egg the first furrow has cut off not more than one-third of the egg from the rest. I have a number of eggs in the two-celled stage in which the second furrow is deeply marked on the upper pole, but extends only about half way around the egg (Figs. 1 and 2). I have no eggs that show how the third furrow comes in, or whether it appears before the second furrow is completed. In later stages the upper hemisphere divides more rapidly than the lower, as shown in Figs. 3 and 4. It has recently been found in the large egg of Desmognathus fusca, with a segmentation similar though even more unequal than that of Hylodes, that there is no equatorial furrow; nor is the third furrow equatorial in Triton cristatus, or constant in Salamandra maculosa. Later stages of Hylodes (cf. Figs. 3 and 4) show the smaller cells at the animal pole, the larger, yellower, more yoke-laden cells at the other pole. The next link in the history (Fig. 5) is the stage where the bastopore is closing in over the yolk-plug.

[1] The figures on the two plates are indicated by consecutive numbers, while text figures are marked by a letter of the alphabet.

2. Embryonic Stages.

Among the embryonic stages, I have material for a more complete history, especially in the later development, than the egg-stages have shown. For convenience in naming the stages, I have numbered them in order, although, owing to some breaks in the series, consecutive numbers do not represent equal advances in development. Since the ages of the preserved eggs are not known, I have compared the eggs with figures of embryos that were watched during development, and whose age was noted. The comparison shows that stages VI, XIII, XIV, and XV, correspond nearly with the embryos under observation which were labelled "7-8 days, about 12 days, just hatched, one hour old." As I have indicated, the former accounts of Hylodes have shown that the tree-frog at no time exists as a free-swimming larva, but that it develops enclosed within a membrane, which is not ruptured until the frog has reached a stage essentially like the adult, though sexually undifferentiated. When the gelatinous secretion about the preserved eggs has been removed, the vitelline membrane is found to be a tough transparent envelope through which the embryo can readily be seen. The eggs are of great size owing to a large amount of yolk, and are at first unpigmented. As the small, colorless embryo develops, it encircles the yolk more and more, while, at the same time, the yolk diminishes in quantity.

In stage IV (Figs. 8, 9), the diameter of the egg preserved in alcohol measures a little less than 3 mm., and the embryo is about one-half the length of the circumference. Both pairs of legs are present as knobs at the sides of the embryo; the tail also is scarcely more than a knob, although slightly lengthened and turned to the right or to the left side. In this stage the tail usually covers the anal opening, though the posterior rim of the anus still shows on the side from which the tail is turned away. The primary divisions of the brain are seen, the auditory vesicles are very distinct, and the optic cups are discernible. The embryo lies flat upon the egg so that its contour is scarcely raised above the level of the yolk. In stage III, the tail and legs are shorter than in stage IV, and on either side of the neck of the embryo are four deep parallel grooves, the ectodermic depressions which lie opposite the visceral pouches. In stage II (Fig. 7), the anus is seen at the posterior extremity of the embryo, and the tail is not formed, but the rudiments of both pairs of legs appear at about this stage. The optic vesicles show as diverticula from the brain. In stage I (Fig. 6), the blastopore is just closing, the divisions of the brain are not seen, but the head end of the embryo is broader than the trunk.

As development advances beyond stage IV, the legs of the embryo elongate, the area of the tail increases, and the brain and sense organs are further differentiated. At about stage VI (Fig. 10), pigment appears at isolated points over the dorsal surface of the embryo and in a dermal fold which covers the base of the anterior legs.

In stage IX (Plate II, Fig. 11), the eyes are large and heavily pigmented. The olfactory pits are plainly seen in an anterior view of the head, and are so far apart as to show from the side. The anterior legs lie close to the head (reaching well to the middle of the eyes when looked at from above). They are covered to the foot by the pigmented dermal fold. In some views, the outlines of toes can be seen. The tail is nearly twice as long as the hind leg, and is spread out under the egg membrane in a thin vascular sheet. The axis of the tail divides it into more or less unequal parts, the part lying towards the head end of the embryo being smaller than that lying on the opposite side of the axis. The tail is not pigmented, but the pigment in the embryo has increased in density, and is extending over the yolk.

Between stages IX and XII, the egg as a whole increases in size, and the embryo manifestly develops at the expense of the yolk. It is possible to see the increasing complexity of the brain until a period when the pigment renders the skin opaque. The fore-legs, as they increase in length, grow around the head (meeting ultimately under the chin), and the dermal fold, by which they have been partially covered, recedes. The hind-legs grow around the yolk, until they almost touch the fore-legs, and the fingers and toes become jointed and provided with suckers. The area of the tail increases. By the time stage XII (Plate II, Fig. 12) is reached, the embryo with the exception of the tail is uniformily though not deeply pigmented; in the tail, there are only a few scattered pigment cells. The lower lip, or rather the chin, is very thick, so that the head is less flat than before; on the upper lip is a single median black horny protrusion or beak.

In stage XIII (Plate II, Fig. 13), the frog no longer appears to be encircling an egg, but what yolk remains is incorporated in the body. Near each thigh is a pale triangular area, raised slightly above the surrounding surface. This is seen again in XIV (Fig. 14), where similar raised patches show on the sides of the neck.

The embryo in stage XIV has hatched, and looks like a somewhat bloated miniature adult frog. It measures 2.3 cm. in length; the adult frog measures 5 to 6 mm. The boundaries of the lymph spaces appear as pale lines in the skin, forming two seams on each side of the embryo and a triangle around each fore-leg, joined by two seams near together

across the ventral surface. The chin is still very thick, and the hard tip of the upper lip persists. The tail is leaf-like and of considerable size, though much smaller in proportion to the body than in previous stages. The earlier observers record that the tail disappears during the first day, or even within a few hours after hatching. The pigment of the embryo in stage XIV is much denser than in preceding stages, but is not arranged in the pattern characteristic of the adult.

Stage XV differs externally from XIV in that the tail is reduced to a small thick knob.

If Hylodes be compared in external appearance with most of the forms of the group to which it belongs, with Rana, for example, it is seen to differ from the outset and throughout the immature stages much more than in the adult stage. The egg differs not only because of its great size, but in the absence of pigment. When the embryo of Hylodes first appears, it is curved ventrally, like the embryo of a Ganoid, over the bulky yolk, and obviously never has a dorsal flexure. When it begins to show its true affinities, it resembles, in the shape of the head, in the prominence of the eyes and in the presence of all four extremities, the frog stage of Rana, rather than the larval tadpole. Only the holoblastic cleavage and presence of the tail betray its relation to the larval form. In short, the usual Anuran metamorphosis is replaced in Hylodes by a direct development. The same fact is borne out by the internal characters.

II. ORGANOGENY.

The organogeny has been studied to some extent from dissections, but for the most part from sagittal and cross sections. In so-called "cross sections," the embryo, on account of its curvature about the yolk, is not everywhere cut transversely; the preparations have purposely been cut in various planes through the lateral axis of the egg, in order to get transverse and horizontal sections of different parts. (The left side of the figure is in each case the right side of the embryo.)

Owing to the large quantity of yolk in the younger embryos, it has been difficult to prepare good series of sections. Eggs killed in Perenyi's fluid are more readily saturated with paraffin and cut better than those killed in picro-sulphuric acid or picric alcohol, but the most satisfactory preparations have been obtained from specimens preserved in a fixative containing picric acid and (after dehydration) left in cedar oil for about 12 hours, in cedar oil and paraffin about 5 hours, and in paraffin (at a melting point of 49° C.) about 8 hours. The sections can be cut of any thickness from 5μ upwards. They were stained on the slide, because it is difficult to orient the embryo in the microtome after surface staining.

Eggs that are well preserved in formalin, make fairly good sections and can be readily cut after two or three hours in paraffin.

1. Origin of Organs.

My material is insufficient to more than suggest a few points in regard to the origin of the organs.

Sections of an egg of the stage of Fig. 5, showing the thick lips of the blastopore and the large yolk plug, and sections of a later stage, where the yolk plug is reduced and receding, are similar to sections of the corresponding stages of Rana. In sections of stage I (Fig. 6), the neural plate is solid, recalling the condition in Teleosts and bony Ganoids.

The notochord appears in a few sections in the posterior region of an embryo of stage II as an evagination of the dorsal wall of the archenteron (Fig. A, n) and the same condition is indicated in younger eggs. Anterior to the section shown in Fig. A the entoderm is continuous over the archenteric cavity, ventral to the notochord, but posterior to this section the entoderm does not appear to be continuous ventral to the notochord.[2]

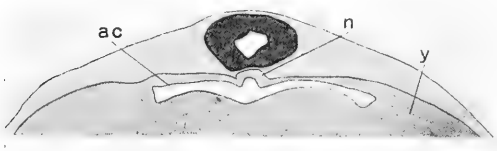

Fig. A. Cross section of stage II, through the posterior part of the embryo, to show the origin of the notochord. ac, archenteric cavity; n, notochord; y, yolk-mass.

2. Digestive Tract.

a. Alimentary Canal.—In stages midway between the first and last of the series, the chief organs derived from the archenteron are definitely established, and the alimentary tract is clearly divided in relation to the yolk-mass, into three regions, anterior, posterior and middle (see Fig. F).

At an early stage, the broad low archenteric cavity is continuous from one end of the embryo to the other (stage II, Fig. B1, ac). The floor of the cavity is the enormous yolk-mass, which is met abruptly at the sides by the thin roof of entodermic cells filled with yolk.

In the same stage (II, Fig. B1), the mesoblast is beginning to separate into splanchnic and somatic layers; as the process continues and the cœlom (Fig. B1, 2 bc) increases, the lumen of the archenteron becomes narrower (Fig. B2, ac), and in the middle region it is completely oblit-

[2] Cf. Bufo lentiginosus, where the conditions are similar, except that one layer only is contributed by the chorda-entoderm to the notochord in the middle region of the embryo.—King, 03.

erated when the splanchnic layers of the two sides have met and form the mesentery which suspends the now solid yolk-mass (Fig. B3, m). After stage V, the yolk-mass in this condition occupies the region of the embryo between the more differentiated anterior and posterior portions of the gut (Fig. F, stage VI, cf. Fig. C, stage VIII).

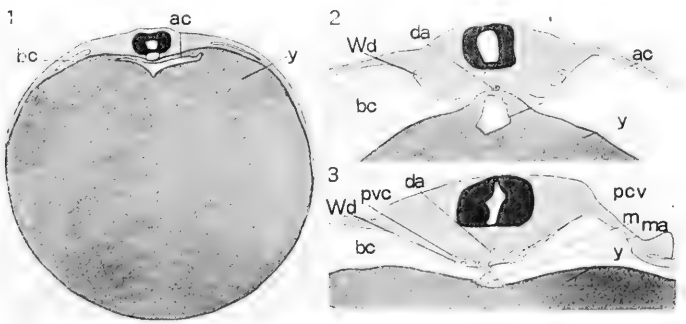

FIG. B. Cross sections of three stages, to show the fate of the archenteric cavity. 1, stage II (drawn to smaller scale than 2 and 3); 2, stage IV; 3, stage VI. ac, archenteric cavity bc, body cavity; da, dorsal aorta; m, mesentery; ma, mesenterial artery; pcv, posterior cardinal vein; pvc, posterior vena cava; Wd, Wolffian duct; y, yolk-mass.

There are no intermediate stages between I and II to show the fate of the blastopore and the history of the anus. In the posterior region of an egg younger than stage I, before the notochord is formed, and in stages I and III, posterior to the notochord, there appears a dorsal evagination of the archenteron, which suggests the neurenteric canal, though not actually traced into the neural tube in the stages in which it is hollow. In stage II the anus is formed, and in IV the differentiation of the hind-gut is in progress. The lateral walls of the posterior part of the broad low archenteric cavity covered by the splanchnic mesoderm fold under until they meet over the yolk-mass (Fig. D1, 2, ac, g). The process of infolding appears to take place from behind forwards, forming a tube that opens posteriorly by the anus, and anteriorly is continuous with the archenteric cavity of the middle region. Thus the cloaca, the portion of the gut from the anus to the opening of the Wolffian ducts, is established, and through all stages retains its lumen.

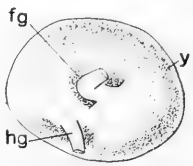

FIG. C. Yolk-mass of an embryo of stage VIII, after the embryo has been taken away, seen from the dorsal side, to show the relation between the yolk-mass and the anterior gut and posterior gut. fg, foregut; hg, hind-gut; y, yolk-mass.

As the cloaca is formed, the lumen of the archenteron is being obliterated in the middle region, and in later stages, the cloaca leads to a rod

of yolk-cells, usually solid[3] at every point except near the middle yolk-mass. The rod, little by little, from behind forwards, is separated from the central mass of yolk, and covered ventrally by the splanchnic mesoderm (Fig. E, F, g). A continuation of the mesentery which slings the yolk-mass in the middle region supports the anterior portions of the rod or gut (Figs. B3, E1, m); but no mesentery is present dorsal to the cloaca.

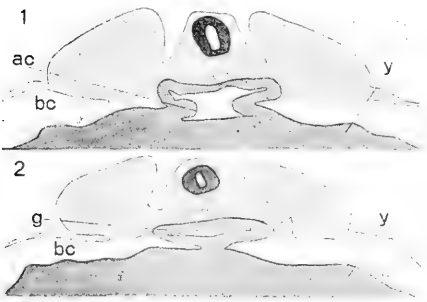

FIG. D. Cross sections of stage IV, to show the formation of the posterior gut; 1 anterior to 2 (and posterior to A2). ac, archenteric cavity; bc, body cavity; g, gut; y, yolk-mass.

The posterior gut at stage VII has been separated from the yolk and completely surrounded by the mesentery as far forward as the level of the anterior limit of the hind legs. The gut up to this time lies in the median line. After stage VII, additions to the gut are still made from the dorsal part of the yolk-mass, but on the left of the median line (cf. Fig. C, hg). As the process goes on, the gut necessarily encroaches upon the middle region.

The first steps in the development of the anterior end of the digestive

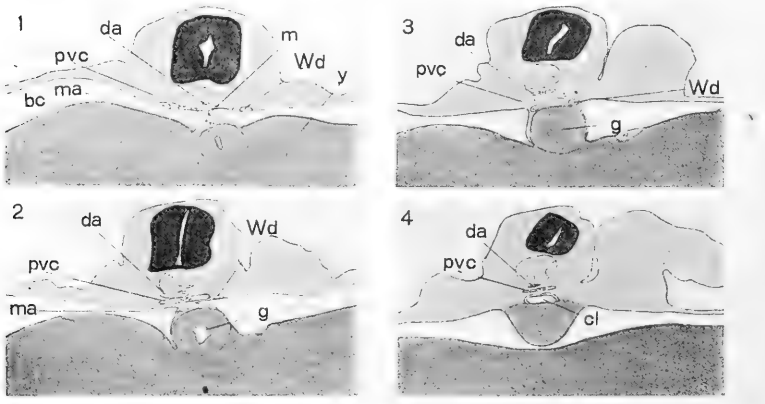

FIG. E. Cross sections of stage VI, to show the connection between the posterior gut and the yolk. 1 to 4 from before backwards (posterior to Fig. B3). bc, body cavity; cl, cloaca; da, dorsal aorta; g, gut; m, mesentery; ma, mesenterial artery; pvc, posterior vena cava; Wd, Wolffian duct; y, yolk-mass.

tract are not apparent in my series. In stage II the anterior portion of

[3] I have seen sections of the tadpole of Rana, in which the archenteron at an early stage is closed for a short distance.

the archenteric cavity is very large. In III the mouth cavity and the pharynx are already formed; posterior to the pharynx the walls of the archenteron, covered by splanchnopleure, are folding under from the sides. Thus the œsophagus is formed, and in stage III is ventrally entirely separated from the yolk-mass (cf. stage V, Fig. G2), but leads posteriorly into the archenteric cavity of the middle region. After stage V, the œsophagus being full of yolk, is for a time nearly solid (stages VI and VII), after the mesentery is first completed. The distinction between

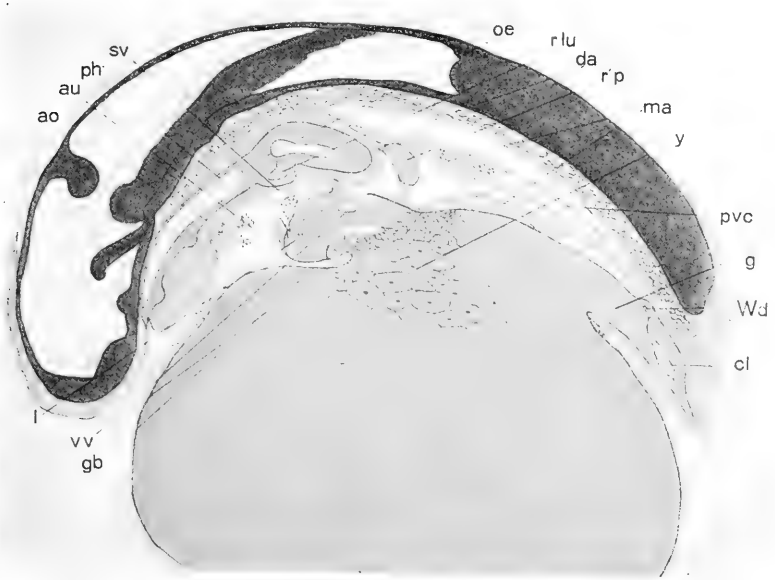

FIG. F. Sagittal section of stage VI. ao, aorta; au, auricle; cl, cloaca; da, dorsal aorta g, gut; gb, gall bladder; l, liver; ma, mesenterial artery; oe, oesophagus; ph, pharynx pvc, posterior vena cava; rlu, rudiment of lung; r'p, 1st rudiment of pancreas; sv, sinus venosus; vv, vitelline vein; Wd, Wolffian duct; y, yolk-mass.

stomach and intestine is not apparent, except that the lumen is greater in the definitive stomach; the intestinal part, near its connection with the yolk-mass in thrown into a loop (Fig. H8, g).

Liver.—In stage III, when the lateral walls of the archenteron are folding under to form the gut, the archenteron is extended into a diverticulum, from a portion of which the liver is subsequently formed. Owing to the large amount of yolk in the egg, the yolk-mass projects beyond the head of the embryo, and so it happens that the diverticulum extends from the gut portion of the archenteron in an anterior as well as a ventral direction, following the curve of the yolk (see Fig. I). In stage

IV, folds are formed in the anterior or more strictly speaking dorsal wall of the diverticulum, in that portion of the diverticulum which lies nearest to the archenteron and immediately posterior to the heart. The liver develops from the folds and the lumen of the more anterior part of the diverticulum becomes obliterated. With the mesoderm covering the folds, blood-vessels are carried into the substance of the liver, and between the two deepest folds runs a large vitelline vein, which leads directly into the posterior end of the heart (see horizontal section of stage IV, Fig. G1, rl, vv). The heart through several stages, abuts against the anterior

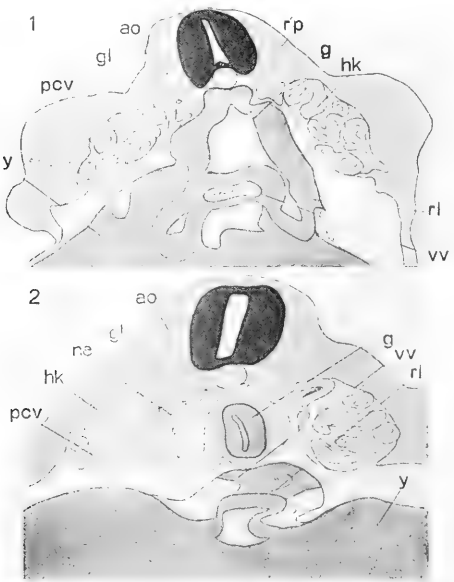

FIG. G. 1, horizontal section of stage IV; 2, cross section of stage V. ao, aorta; g, gut; gl, glomus; hk, head-kidney; ne, nephrostome; rl, rudiment of liver; r'p, 1st rudiment of pancreas; vv, vitelline vein; y, yolk-mass.

end of the liver, and as the liver differentiates, the spaces filled with blood between the trabeculæ, are in direct communication with the sinus venosus (Fig. F, 1 and sv).

As the liver develops, it is surrounded by the splanchnopleure and is suspended from the dorsal wall of the body cavity; the portion of the gut from which the stomach develops lies to the left of the mesentery thus formed (Fig. H2, 3). The communication between the liver and gut is gradually reduced to a small opening (stage VII), the definitive bile-duct, which enters the gut close to its connection with the central yolk-mass. As the gut is lengthened at the expense of the yolk-mass, the

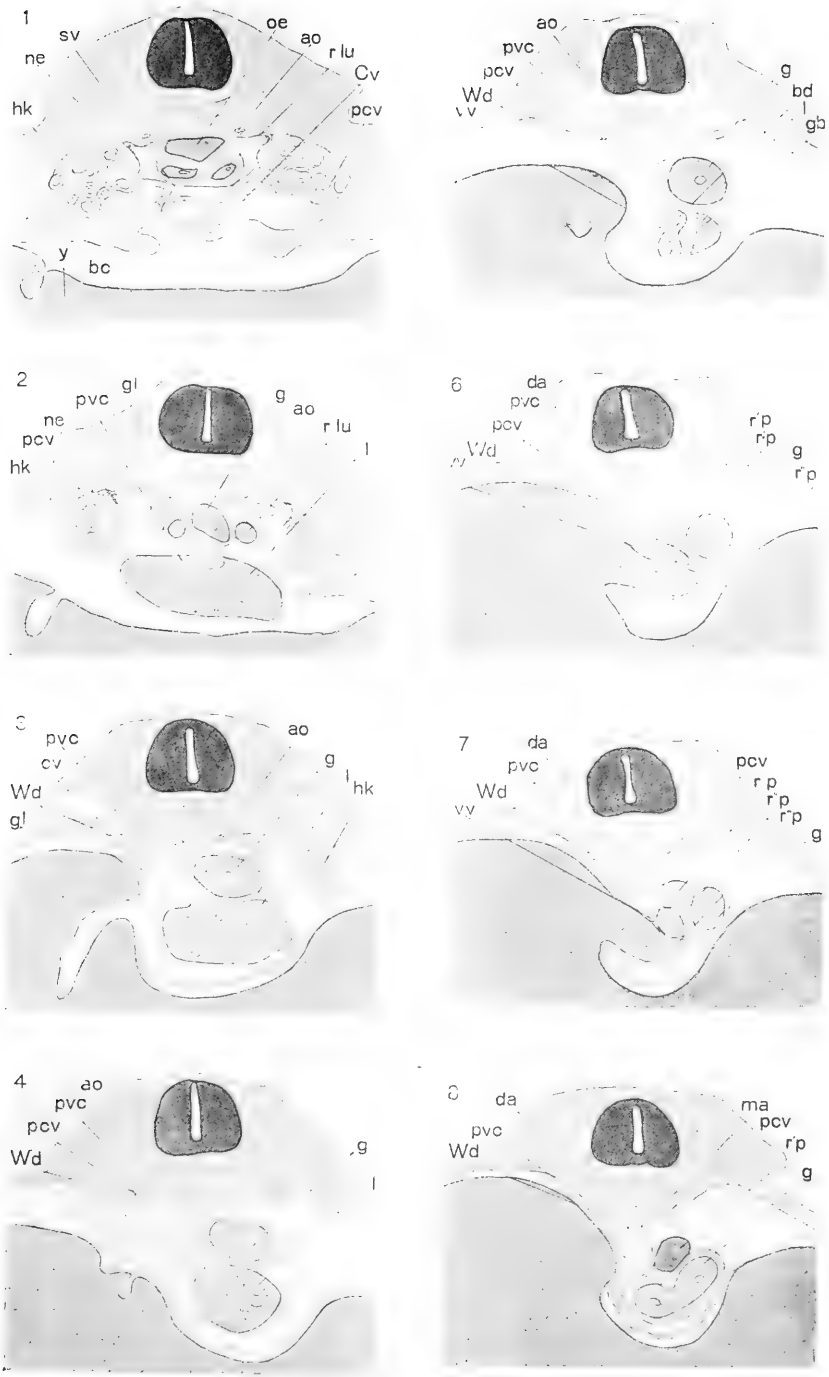

FIG. H. Cross sections of stage VIII. 1-8 from before backwards. ao, aorta; bc, body cavity; bd, bile duct; Cv, Cuverian vein; da, dorsal aorta; g, gut; gb, gall bladder; gl, glomerulus; hk, head-kidney; l, liver; ma, mesenterial artery; ne, nephrostome; oe, oesophagus; pcv, posterior cardinal vein; pvc, posterior vena cava; rlu, rudiment of the lung; r'p, r''p, r'''p, 1st, 2d, 3d rudiment of the pancreas; sv, sinus venosus; vv, vitelline vein; Wd, Wolffian duct; y, yolk-mass.

yolk recedes from the region where the liver is established (cf. Fig. F with Figs. G and H 4-8). The lengthened gut forms a loop posterior to the liver and joins the yolk-mass on the right side. The bile-duct is increased in length and in stage VIII still opens into the gut near the point where the gut joins the yolk-mass (see Fig. H5, 6); since the gut has lengthened, it seems that the entrance into it of the bile-duct has shifted toward the yolk. The gall-bladder appears about stage VI as a diverticulum from the ventral wall of the bile-duct close to the liver (Fig. F, gb. cf. Fig. H5).

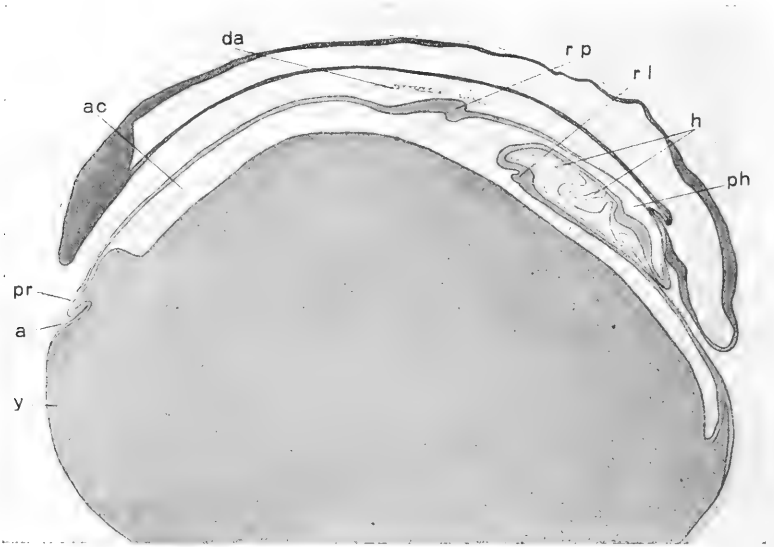

FIG. I. Sagittal section of stage IV (nearly in the median plane). a, anus; ac, archenteric cavity; da, dorsal aorta; h, heart; ph, pharynx; pr, proctodaeum; rl, rudiment of liver r'p, 1st rudiment of pancreas; y, yolk-mass.

Pancreas.—The pancreas develops from three rudiments. The first in time of appearance is present in stage IV as a diverticulum from the dorsal wall of the gut (Fig. G1, I, r' p). Anteriorly this rudiment ends in a solid mass of cells containing much yolk, and entirely separated from the gut. The connection between the gut and this rudiment is already lost in the next stage.

Early in stage VI, a second rudiment appears as a diverticulum from the dorsal wall of the definitive bile-duct nearer to the gut than to the liver (cf. a later stage Figs. H6, 7, r'' p). The third rudiment arises later, in the same region as the second, but from the ventral wall of the duct (cf. Fig. H6, 7, r''' p). The connection with the definitive bile-duct is re-

tained where the second and third rudiments originate. No trace remains of the place of evagination of the first rudiment from the gut itself.

Mouth.—The stomodæum is from its first appearance (after stage IV) slit-shaped, and lies well under the ventral surface of the head (cf. Fig. 15, Plate II). A free passage from the stomodæum into the mouth cavity is not established until stage X.

A noteworthy fact in the development of the mouth is the entire absence of horny jaws, and teeth, and of fringed lips. The hardened tip of the upper lip which develops late in embryonic life will be described in another place: it has not the characteristics of a tadpole tooth. It may be here noted that an adhesive gland is also wanting. The tongue is first found in stage VII.

Visceral Pouches.—The visceral pouches first appear in the series in stage III. It has been noted that, in surface view, there are four ectodermic depressions. In section, it is found that the hyo-mandibular pouch is a deep diverticulum from the wall of the pharynx and that its entodermic lining in many cases lies in contact with the outside ectoderm; the first branchial pouch is in almost the same condition, but not in actual contact with the ectoderm; the second branchial pouch is merely a shallow diverticulum, and the third barely begun. In stage IV (Fig. J), the same conditions exist, except that the third branchial pouch has become deeper. After stage IV the pouches are gradually reduced, and before VIII have almost disappeared.

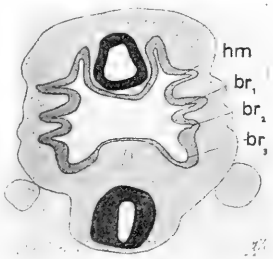

FIG. J. Horizontal section through the branchial pouches in stage IV. br 1, br 2, br 3, 1st, 2d, 3d branchial pouch; hm, hyomandibular pouch.

Not one of these pouches in any preparation can be demonstrated to open to the outside, hence the evidence as far as it goes shows that no true gill-slits are formed in the embryo of Hylodes. Before this conclusion can be placed entirely beyond doubt, it is necessary that a more complete series of embryos be examined.

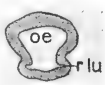

FIG. K. Cross section of the oesophagus and rudiment of the lung in stage V. oe, oesophagus; rlu, rudiment of lung.

The statements of earlier writers in regard to the gills have been entirely confirmed. Not a trace of either external or internal gills can be found in any of the embryos. It will be shown later that the circulation in the gill arches is extremely simple.

Œsophagus and Lungs.—The lungs are derived from a single rudiment which arises in stage V by a constriction of the ventral portion of the œsophagus from the dorsal (Fig. K). As the rudiment becomes more

completely separated from the gut, the œsophagus is flattened dorso-ventrally, and loses its lumen (Fig. L). The solid portion of the œsophagus extends for a little distance anterior and posterior to its union with the lungs (Figs. L1, 2 and H1, œ, rlu). The lung rudiment lengthens and becomes paired posteriorly.

The foregoing description shows that by the time stage VIII is reached, the principal adult organs derived from the archenteron are well differ-

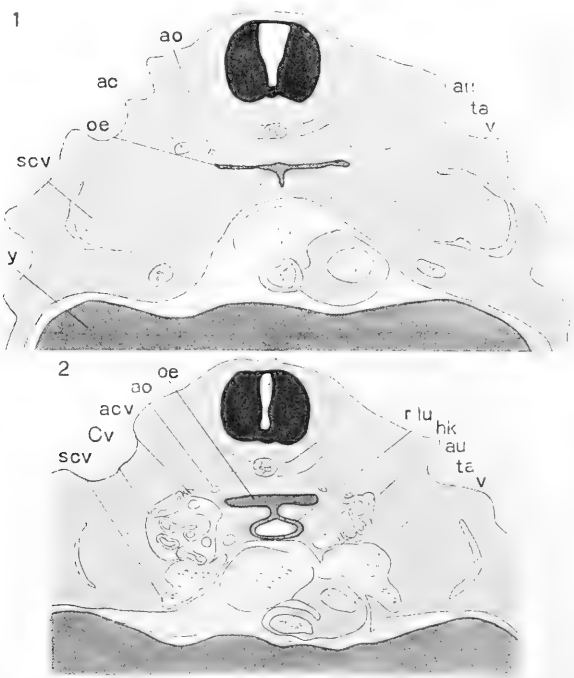

Fig. L. Cross sections of stage VIII. 1 anterior to 2 (both anterior to Fig. H1). acv, anterior cardinal vein; ao, aorta; au, auricle; Cv, Cuverian vein; hk, head-kidney; oe, oesophagus; rlu, rudiment of lung; scv, subclavian vein; ta, truncus arteriosus; v, ventricle; y, yolk-mass.

entiated. The liver, after stage VIII, becomes more compact; it is less intimately connected with the heart, and definite efferent hepatic vessels are formed. The gall-bladder increases in size and after stage X contains a clear, yellowish substance. The three rudiments of the pancreas become unified into a compact organ. The lungs increase in size, and soon after stage VIII hang freely in the body cavity, surrounded by the splanchnopleure. Their walls and the walls of the œsophagus are somewhat pigmented after stage XII. The lungs, even as late as the time of

hatching, have attained no great size and are remarkably simple in structure. In sections of the late stages, and in adults preserved in alcohol, they appear as hollow thin-walled sacs, not showing the trabecular structure of the lung of the common frog. In all sections of late embryonic stages, the walls of the lungs are more or less wrinkled, so that in life they may have been capable of expansion to some extent; in their present condition, however, they extend, at most, only slightly beyond the anterior limit of the liver.

Differentiation of the alimentary canal, after stage VIII, is accompanied by the disappearance of yolk from its walls, and by the absorption of the large yolk-mass that still separates the anterior part of the intestine from the posterior (cf. Figs. B1, and O for the size of the yolk relative to the embryo). The yolk first disappears from the walls of the pharynx. In closed portions of the gut, the lumen as a rule reappears immediately upon absorption of the yolk, but the roof and floor of the broad low œsophagus remain pressed together, even after there is no yolk remaining in them, and the opening to the lungs is not re-established until after stage XII. The stomodæum is open into the entodermal buccal cavity at stage X (as already stated), so that after XII, there is free communication from the lungs to the outside. Posterior to the lungs, the walls of the œsophagus, after the reduction of the yolk in their cells (stage XII), become convoluted but no lumen exists until after hatching. The convolutions may be partly due to shrinkage in the preparations, although in an older specimen where food has entered the stomach, the walls remain distended after preservation; this seems to indicate that the walls remain collapsed until food has passed through the gut. The stomach portion of the gut retains its lumen from the time of its formation. The intestinal portion continues to be lengthened after stage VII by additions from the yolk, has a small lumen, and becomes convoluted about stage XII. The walls of the posterior gut lose their yolk first in the cloaca, then in the portion near the yolk, and lastly between these parts. As in the anterior gut, the walls become convoluted in stage XII. The lumen near the middle yolk is enlarged in stage XII and extends through the gut for some distance posterior to the yolk.

Since the posterior gut is constantly added to from one side (the left) of the yolk, and the anterior from the other side, it comes about that the two connections between gut and yolk pass each other, and a middle region between them does not exist after stage XII in the same sense as before. Still there remains a large amount of yolk, which after stage XI does not retain its solid condition (see Fig. M), and is no longer reduced by gradual contributions to the more differentiated parts of the gut.

In stages XI to XII, the interior of the yolk is split in various places, and the fissures contain a substance which stains deeply with carmine. The substance may be the product of cells that appear to be breaking down in the borders of the fissures. In the next stage (XIII), the fissures have formed a continuous cavity that leads into the anteriorly and posteriorly differentiated gut, thus completing the lumen of the alimentary canal. In the meantime, the whole yolk becomes lobed, and large portions are contributed to the gut at once. This takes place with almost uniform regularity; the first lobe is formed in stage XII, and from that part of the yolk which immediately joins the anterior gut; the second is added from the yolk in connection with the posterior gut. The amount of yolk is con-

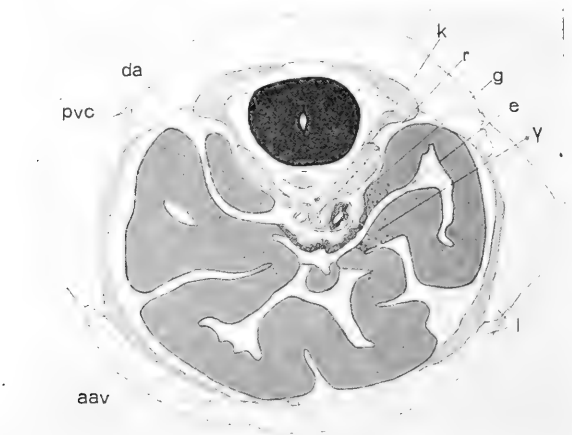

FIG. M. Cross section of stage XIV. aav, anterior abdominal vein; da, dorsal aorta; e, differentiated portion of entoderm; g, gut; k, kidney; ls, lymph spaces; pvc, posterior vena cava; r, reproductive organ; y, entodermic cells laden with yolk.

stantly reduced in the large lobes, and finally in stage XIV the yolk is found in only a small portion of the intestine (cf. Fig. M).

A comparison of Figs. B1, 2, 3, Fig. M, Fig. C and Figs. I, F, O, will show briefly, in review, the main facts in the history of the yolk-mass and its relation to the embryo.

There is no coiled intestine as in the vegetable feeding larva of Rana, but the yolk holds the same relation in the digestive tract as it does in two other yolk-laden Amphibian eggs, those of Plethodon cinereus and Ichthyophis glutinosus (cf. Fig. B3 with Montgomery '01, Fig. 5 and Sarasin '87, Fig. 7, and cf. Fig. N with Sarasin '87, Fig. 8). Very much the same relations appear from the descriptions to exist in Alytes obstetricans at one period, although in this form a coiled intestine is later developed.

From the earliest stages of the series, the entire yolk is divided into cells, which are larger in the center and smaller at the periphery (Fig. F). The cell walls are sometimes not clear in poorly preserved eggs of early stages.[4] The cells are, in all stages, smallest in that part of the yolk where the anterior and posterior parts of the gut are forming. In later stages while the yolk is disappearing from the gut walls, it is lost also in the cells of the middle yolk-mass immediately beneath the mesentery and by the time the cavity of the alimentary canal is established in the central yolk-mass, the wall beneath the mesentery is differentiated into a layer of small cells that pass abruptly into huge yolk-laden cells several times their own size (Fig. M, g, e, y).

The frog may feed before the yolk is entirely absorbed, for the stomach of a hatched frog contains green food, while part of the intestine is still loaded with yolk.[5]

3. Heart and Blood-Vessels.

The details in the development of the blood-vessels have not been worked out, but the chief events in their history have been determined.

Heart.—The heart develops from the mesoderm ventral to the pharynx.

In stage IV, it has arrived at the condition of a bent tube (Fig. I) with a slight constriction between the auricular and the ventricular parts. It undergoes the usual changes of further twisting, thickening of the ventricular walls, etc. The longitudinal septum in the truncus appears at about stage VII, but the septum between the auricles, which in the frog appears simultaneously with the septum of the truncus, is not found in Hylodes until stage IX; it attains no great size until XI, after which it separates a very small left auricle from the right. The relatively late development of this septum may be correlated with the slow growth of the lungs and consequently of the pulmonary circulation (cf. p. 493).

Veins.—For a full understanding of the vitelline circulation, the examination of living material is essential. From the preserved preparations no constant arrangement of vessels on the yolk can be determined. In stage IV, the yolk is literally covered with great vessels, in which are large blood corpuscles filled with yolk granules; the vitelline blood is collected in a large vein, which passes through the liver, and constitutes in early stages the posterior end of the heart (Fig. G1, vv). After stage VI,

[4] It is stated that the yolk-mass of Desmognathus fusca and of Ichthyophis is at one period segmented only peripherally, and that in Plethoden cinereus also the boundaries of the yolk-cells disappear.

[5] The embryo of a viviparous salamander will feed although yolk is still contained in the digestive tract.

the vitelline vein comes from the right side of the yolk (where the liver, yolk and gut are connected), passes along the dorsal wall of the liver, and joins the posterior vena cava before it enters the heart. Lastly after about stage VIII, the vitelline vein (Fig. H7-4, vv) is greatly reduced and no longer passes through the liver as a distinct vessel, but is lost in the tissues of the organ.

The Cuverian veins, the anterior cardinals, and perhaps the posterior cardinals are present in stage IV (cf. Fig. N, with the following de-

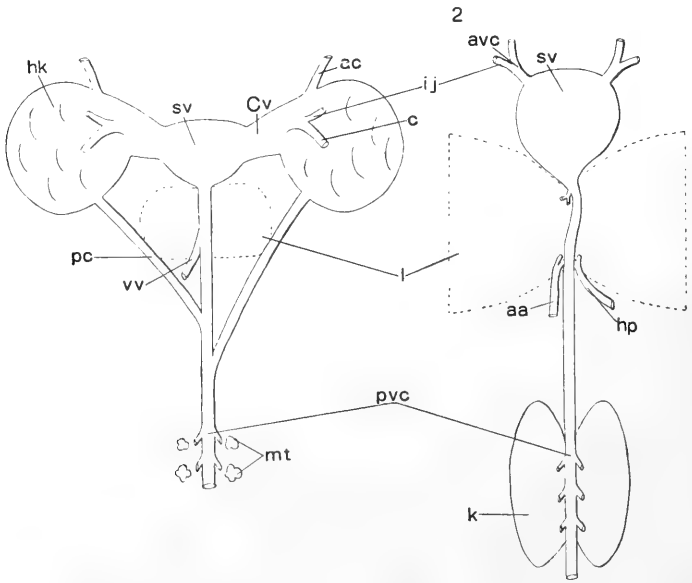

Fig. N. Diagram of veins. 1, stage VIII. 2, stage XV. aa, anterior abdominal vein; ac, anterior cardinal vein; avc, anterior vena cava; c, cutaneous vein; Cv, Cuverian vein; hk, head-kidney; hp, hepatic portal vein; ij, inferior jugular vein; k, kidney; l, liver; mt, mesonephric tubule; pc, posterior cardinal vein; pvc, posterior vena cava; sc, subclavian vein; sv, sinus venosus; vv, vitelline vein.

scription). There is also, on each side of the yolk, an enormous cutaneous vein, which lies in the somatopleure, hence not directly in contact with the yolk-mass, and passes beneath the forelegs into the Cuverian vein. It is so conspicuous from stages VI to IX, that it deserves especial mention as an embryonic vessel (see Fig. L1, 2, scv). From its position the cutaneous vein would seem to be the forerunner of the subclavian vein, but it has not been seen after stage XII.

The posterior cardinals are evident after stage V. They diverge from a common trunk (2 sections posterior to Fig. H8), and passing an-

teriorly, bathe the head-kidneys and empty into the Cuverian veins (Fig. H8-1, pcv, cv). The posterior cardinals are remarkably conspicuous from stages VI to IX, and persist to stage XII, when only vestiges of them can be found posterior to the degenerated head-kidneys.

The anterior cardinals (Fig. L, acv) also empty into the Cuverian veins. After the degeneration of the posterior cardinals, the Cuverian veins and anterior cardinals persist as the anterior venæ cavæ. After stage VII, the inferior jugular veins from the ventral region of the neck enter the Cuverian veins with the cutaneous, and persist after the cutaneous veins have been reduced. In stage XV, they are present as branches of the anterior venæ cavæ.

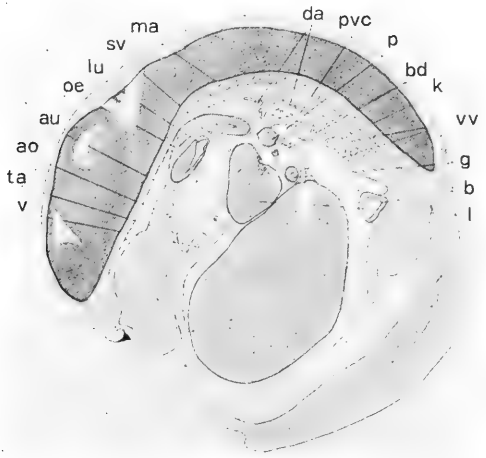

FIG. O. Sagittal section of stage XII (drawn to same scale as Fig. B1). ao, aorta; au, auricle; b, bladder; bd, bile duct; da, dorsal aorta; g, gut; k, kidney; l, liver; lu, lung; ma, mesenterial artery; oe, oesophagus; p, pancreas; pvc, posterior vena cava; sv, sinus venosus; ta, truncus arteriosus; v, ventricle; vv, vitelline vein.

The posterior vena cava appears at about stage VI, as a median vein, which passes along the dorsal wall of the liver, receives the vitelline vein, and empties into the sinus venosus (cf. Fig. H7-2). As the vitelline vein becomes less prominent the vena cava increases in importance, and takes its place as the posterior end of the sinus venosus. In later stages, as the heart becomes separated from the liver, the posterior vena cava is also separated from the liver (Fig. O) and receives definite efferent vessels from it. The posterior vena cava joins the right posterior cardinal (Fig. H6, 7, pvc, pcv) at a little distance anterior to the union of the two cardinals; and after the posterior cardinals disappear, continues to

hold the same course. As the posterior vena cava, where it joins the heart, acquires greater importance than the vitelline veins, and replaces the posterior cardinals in the trunk, it becomes the principal vein posterior to the heart. It receives numerous branches from Wolffian bodies, and is the vein of the very vascular tail.

The hepatic portal vein cannot be distinguished before stage IX. The vitelline vein joins it at the liver, and is its most conspicuous branch until stage XII.

The anterior abdominal vein has been first observed in stage XIII. It runs in the median line (cf. Fig. M, aav), from the posterior end of the embryo, along the ventral surface of what remains of the yolk, bends back around the anterior limit of the yolk, passes posteriorly for a short distance in the pericardium and enters the liver with the hepatic portal. The stages are wanting to determine whether the anterior abdominal has a paired origin, and whether when it first develops, it opens directly into the sinus venosus. In stages XIV and XV, when the yolk has gone from the region of the heart and liver, the anterior abdominal passes directly from the ventral wall of the abdomen into the liver, making no loop anteriorly as in stage XIII. The vesical vein, a branch of the anterior abdominal from the bladder, is found in stage XIV.

Arteries.—The dorsal aorta in stage IV is fully established; it is formed by the union of the aortic arches and extends to the posterior end of the embryo. It might fairly be expected that so large a quantity of yolk as the embryo possesses would be supplied by a fixed arterial system, but it has been impossible to trace any definite arteries to the yolk-mass at this stage. It can only be said that the infolding walls of the posterior gut are surrounded by blood corpuscles, and there is some evidence that a branch from the aorta connects with this region.

In stage VI, a branch of some size passes from the aorta into the mesentery which slings the yolk of the middle region (Fig. F, B3, ma); thence it runs posteriorly along the rod of yolk, and posterior gut (Fig. E, ma), and probably gives off branches on either side, to the yolk. The vessel, reduced in size, persists in later stages as the cœliaco-mesenteric artery (Figs. H8, O, ma). The point where it leaves the dorsal aorta is slightly posterior to the union of the two aortæ, and in the region between the union of the posterior vena cava with the right and with the left posterior cardinal. The point of union with the left cardinal is, as it were, thrown back to admit of the passage in the mesentery of this little branch of the aorta. The arterial system in the visceral arches has been worked out as far as possible in the present series; but it is hoped, as in the case

of the visceral pouches, that the observations may be amplified by the study of more abundant material. The vessels of the arches are characterized by extreme simplicity. The first vessel to be completed is the aortic arch of the first branchial arch in stage IV; very soon the vessel of the second arch is also complete. In the meantime incomplete vessels or lacunæ are found in the mandibular and hyoid arches, but at no time is there a complete vessel from the truncus to the collecting aorta in any visceral arch save in the first and the second branchial.

The pulmo-cutaneous vessel when first seen in the series, at about stage V to VI, branches near the truncus from the aorta of the second branchial arch. A study of fairly abundant material shows no indication of the origin of the pulmo-cutaneous from an artery of the fourth branchial arch, as in the frog. There is no representative of the vessel of the third branchial arch.

The condition then in stage VI (Fig. P) is as follows:—The first branchial arch passes from the truncus into the collecting aorta, and is continued anteriorly as the carotid. The aorta of the second branchial arch passes posteriorly as the systemic, meeting the corresponding vessel of the other side, posterior to where each has supplied the glomus of the head-kidney. The collecting aorta is already somewhat reduced between the dorsal parts of the first two arches. Lacunæ are still found in the mandibular and hyoid arches; the vessel in the hyoid is connected with the ventral portion of the aorta of the first branchial, but the aorta is not complete in either mandibular or hyoid arch. Finally, at the point where the first and second branchial arteries separate, a more posterior branch, the pulmo-cutaneous, is given off.

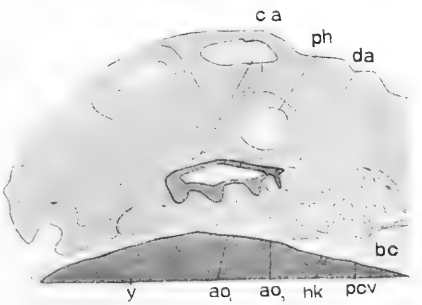

FIG. P. Sagittal section of the head in stage VI. ao_1, ao_2, aorta of the 1st and of the 2d branchial arch; bc, body cavity; ca, carotid artery; da, systemic aorta; hk, head-kidney; pcv, posterior cardinal vein; y, yolk-mass.

In stages soon after VI, as the visceral arches are obscured, the collecting aorta between the carotid and systemic degenerates, and the embryonic arrangement of the aortic arches cannot be detected; the carotid, systemic and pulmo-cutaneous arise as three great branches from the truncus.

Pulmonary circulation.—The study of the pumonary vessels has given mostly negative results. The cutaneous artery is an established vessel from stage VI on, but in the same section where it can be clearly traced, nothing can be seen of the pulmonary artery in specimens younger than

the stage of hatching. In a longitudinal section of stage XV, it appears as a branch of the cutaneous vessel. The pulmonary vein has not been seen with certainty in any stage. As other vessels are clearly seen in the preparation, it follows with great probability that the pulmonary vessels are very small or wanting in embryonic stages. It will be remembered that the lungs are small, and also that the left auricle is very small, and the septum between it and the right auricle is late in developing.[6] All the facts then in connection with the morphology of the organs for pulmonary respiration, go to show that the lungs are not of great functional importance in embryonic stages.

Lymph sinuses.—Sub-dermal lymph sinuses appear about stage X as narrow spaces; by stage XIII they are enlarged into enormous sacs that completely surround the animal (cf. Fig. M). They are separated by narrow partitions, the principal sinuses being a dorsal, a ventral, and lateral chambers.

4. EXCRETORY AND REPRODUCTIVE ORGANS.

The excretory system of Hylodes has been described by Selenka, **82**, from a reconstruction of one stage (about VI, judging from the figure independently of the excretory system). He has added notes on a later stage (about XIII). Selenka concludes that the pronephros degenerates and the mesonepronephros appears early as compared with other amphibia, and he associates this condition with the absence of gills, a combination approaching the condition in Reptiles. A comparison of this sort requires further evidence from the study of the function of the organs.

Field, **91**, interprets Selenka's figures and description as showing that the pronephros in the earlier stage appears already in a degenerate condition, the collecting trunk being extended into irregular blind sacs instead of convoluted, and opening directly into the anterior end of the segmental duct. I have not made reconstructions of any stage, so that I cannot judge of the accuracy of Selenka's[7] figures of the model, but in

[6] In some of the lungless salamanders the auricle is small, the septum between the auricles is incomplete, and the pulmonary vein is wanting.

[7] The spinal cord in Salenka's cross section is in the shape of a cross, which does not appear in my sections. His "J," called in the explanation of the figure "Darm," is in the usual position of the heart, while the unlettered space in the shading above it might well be the gut and rudiment of lungs. Selenka mentions the lungs as present in the older embryo but wanting in this. We have seen that the lung diverticulum is present even before VI. I have found the degenerate glomus and rudiments of the ciliated funnels in stages later than those in which Selenka mentions them.

section the tubules appear to be, through several stages including Selenka's early stage, as regular as in sections of Rana. It would be of interest to determine the exact state of the organ from a series of models; but at present, I can only give a brief statement of the time of appearance and duration of the principal parts of the excretory system.

The Wolffian ducts are found in stage II. While the archenteric cavity is still broad, their posterior ends are far apart, but when the gut has been differentiated from the archenteron, they meet in the median line, where they open into the gut. It has already been noted that from this point backward, the gut remains open as the cloaca. It seems to be the enlargement of the gut which Selenka calls the " allantois."

The tubules of the pronephros are already present in stage III in a simple form; in IV, they are much coiled, and before stage VI they form a compact organ. In stage III, the three ciliated funnels open into the peritoneal cavity (cf. Figs. G2, H1, 2, ne), the two anterior funnels close together. In XI, the tubules of the head-kidney are irregularly dilated; in XIII, the organ is reduced in size, and the tubules are solid in part. Vestiges of the ciliated funnels persist until XIV, when their number cannot be determined from the sections. In XV, the degenerate pronephros has almost entirely disappeared. The Wolffian duct remains connected with the head-kidney until stage XV, when only a vestige of it remains anterior to the Wolffian body. The glomus is present in stage IV (Figs. G1, 2, gl), and develops and degenerates at a nearly equal rate with the pronephros, except in later stages, when it is particularly large relatively to the size of the head-kidney. In stage XV a vestige of it still persists.

About four or more mesonephric tubules are present in stage VI, and from that time they increase in number, size and complexity until they have become a compact mass (stage XV, cf. Figs. M, O, k).

The bladder appears at about stage X as a solid projection of the ventral wall of the cloaca. As it develops, it extends anteriorly, becomes hollow and distally bifid (from stage XI), and by stage XIV, it is a thin-walled cleft sac of considerable size (cf. Fig. O, b).

The genital ridges arise at about stage VII, and are well developed in stage XV (cf. Fig. M, r). At that time, there is no appearance of degeneration of the anterior portion into fat bodies, no differentiation into testes or ovaries, nor has the rudiment of the Müllerian duct been seen.

5. TAIL.

The tail of Hylodes calls for particular attention because of the unusual condition of almost every feature of it. In a word, it constitutes not a muscular organ for locomotion but a vascular organ for breathing.

The notochord is the axis of the tail; the flat surfaces are morphologically the right and left sides. Thus the vascular lamellæ of the tail are in the position of the dorsal and ventral fins of the tadpole. To whichever side the tail is turned in the egg the part which lies cephalward from the notochord is dorsal, and the part on the opposite side of the notochord is ventral. These relations are clearly established by the relative positions of the notochord, spinal cord, and dorsal aorta (Fig. Q). While the tail after stage IV becomes broader and thinner, as described from the surface view, the connective tissue becomes spongy, and through its meshes is a network of capillary blood-vessels. The ventral part is more richly supplied with blood-vessels than the dorsal, a condition perhaps correlated with the fact that it contains the dorsal aorta which carries the main supply of blood. The aorta extends with the notochord nearly to the end of the tail; no main efferent vessel runs for any distance through the tail, but the blood is collected in the posterior vena cava near the union of the tail with the body.

Fig. Q. Cross section of the tail in stage VIII. bv, blood-vessels: da, dorsal aorta: m, muscle: n, notochord; sc, spinal cord.

At the base of the tail the diameter of the notochord from stages VI to XII far exceeds its diameter in the body of the embryo. In the tail itself the notochord diminishes in size toward its distal end. The diameter of the spinal chord is greatly reduced as compared with its size in the body, especially in late stages. Surrounding the notochord is a layer of muscle that is connected with the muscles of the body and like them is segmented. No vertebræ are found in the tail.

In stage XIV the notochord, muscles and spinal cord have become reduced, and the tail is more solid but still very vascular; in stage XV when the tail is almost entirely absorbed the tissue is compact.

6. DERMAL FOLD.

The fold of skin, which in several embryonic stages partially covers the arms, will be described in more detail. The anterior leg lies in a space which is the angle between the head of the embryo and the yolk (see figure). Across this space stretches the dermal fold whose free anterior edge reaches from the head to the side of the yolk. The fold is composed of two layers of ectoderm separated by mesoderm. The folds of the two sides are entirely independent of one another. The fold differs from the operculum of the tadpole, in that it is continuous with the epidermis of the body posteriorly, and its free edge is directed anteriorly. But the anterior legs of Hylodes, for some time after their first appearance, are

directed anteriorly from the body because of the large amount of yolk at the sides of the embryo; hence the dermal fold resembles an early stage of the operculum of the tadpole in its morphological relation to the anterior leg.

The entire separateness of the dermal folds of the two sides recalls the condition of the operculum in the two representatives of the Aglossa, Dactylethra and Pipa, where the operculum opens on both sides. In these two forms, however, the anterior legs are not covered by the operculum.

It is not evident from the points of difference and of resemblance between the dermal fold of Hylodes and the operculum of the tadpole whether or not the two structures are homologous.

BEAK.

The black tip on the upper lip is seen at late stages in external views (Plate II). It is triangular in sagittal sections (Fig. 0), appearing in sections at about stage X. The beak does not surmount a column of epithelial cells like the teeth and edge of the jaw of the tadpole. It attains its greatest size in the last stage within the egg, and whether by accident or normally, the tip is sharp in stages XI, XII and XIII, and blunt in the hatched tadpole. The facts suggest that its function is to rupture the egg membrane (cf. Rana opisthodon).

SENSE ORGANS.

I have not attempted to make a study of the sense organs, but observe that they arise in very early stages as in the tadpole.

The auditory vesicles are already seen in stage II. In the same stage, the optic vesicles are in the condition of diverticula from the brain; their connection with the brain is reduced in the next stages, and in III the lenses are being formed. Thickenings of the epidermis, the rudiments of the anterior nares are present in stage III, and the hollow diverticula, reaching anteriorly from the pharynx, are, to all appearances, the beginnings of the posterior nares.

7. COMPARISON WITH OTHER FORMS.

In immature stages of Amphibia, there are various kinds of adaptations to different surroundings. In each of the three living orders, there are exceptions to the rule that the Amphibia develop from eggs laid in water and pass through a free-swimming stage. Among the Urodeles are the viviparous Salamandra atra, also Salamandra maculosa, and the forms with terrestrial eggs (some species of Amphiuma, Plethodon cenereus Antodax lugubris, Desmognathus fusca); among the Gymnophiona

are Typhlonectes compressicauda, and Dermophis thomensis, two viviparous species, Hypogeophis, in which the eggs are terrestrial and the larval life is completed within the egg-membranes, and Ichthyophis glutinosus, with early stages out of the water; and among the Anura are a number of forms recently enumerated by several writers.[8]

The facts as far as they are known in regard to the development of the exceptional forms point to the interpretation that they are descended from forms which originally had aquatic larvæ.

It is to be noted that among Anura, there are no known cases of internal fertilization and viviparity.[9]

It seems clear, from what has been described of the habitats, that a form with a free-swimming larva would not survive in some at least of the regions where the animals are found. Salamandra atra lives in high Alps, where the water runs swiftly. Hylodes and Rana opisthodon also live where there are no pools. In the latter cases, the larva and adult no longer inhabit two different environments, but the embryo has become adapted to the environment of the adult of the original form.

There are other ways in which the difficulties of insufficient water are met. Development may be hurried through (in Lythodytes latrans) in a rain pool, where the supply of water is uncertain, or the tadpoles may be provided for in such a way as to survive a drought, as in Cystignathus mystaceus. In such a case as that of Pipa, in which the eggs are carried on the back, while the parent stays in the water, the advantage of the habit is not obvious at present.

The various adaptations involve in different degrees not only the larvæ but often the instincts and certain structures of the parents (as when pouches are developed).

Whatever the cause or origin of the adaptations may be, it is found that, with a few exceptions, the larvæ or embryos of all the forms still retain some of the organs characteristic of the free-swimming larva. These organs are not, however, always used in the same way, or even for the same purpose as in the aquatic larva. In some cases (and among them Hylodes) all the larval organs are not present, and furthermore some new organs which are not found in the larva occur in the embryonic forms.

The most patent differences in the requirements of the aquatic larva and of the terrestrial or viviparous embryo concern nutrition and respiration. In the embryonic form there is the necessity for a special food

[8] Brandes and Schoenichen, 01. Wiedersheim, 00. Sampson, 00.

[9] In Pipa, fertilization takes place perhaps in the oviduct, which is curiously protruded over the back of the female when the eggs are laid.

supply and for organs of respiration adapted to a medium other than water.

In the viviparous salamander, the larva imbibes the fluid within the uterus in which it swims. In terrestrial forms of each order, the food supply is given to the embryo in the enormous mass of yolk in the egg. In spite of the large size of the egg, the type of cleavage in Hylodes is holoblastic as in other Anura. The yolk-laden eggs of other forms have scarcely been studied. The holoblastic type has been found to occur in Alytes and in Desmognathus fusca (both formerly supposed to have meroblastic eggs).

As would be expected, the number of the eggs is comparatively small in forms where the eggs are excessively large. The absence of pigment is another peculiarity that has been noted in many cases where the eggs are concealed, either carried by the parent or laid in sheltered situations. The following table summarizes the observations, so far as they have been made:

	Color of Eggs.	Deposition of Eggs.	Number of Eggs.	Size of Eggs.
Nototrema marsupiatum.		Dorsal pouch ♀.	200	"Smaller than a pea."
Nototrema oviferum.	Not pigmented.	Dorsal pouch ♀.	15–30	1 cm.
Nototrema fissipes.		Dorsal pouch ♀.	16	10 mm.
Nototrema pygmaeum.		Dorsal pouch ♀.	4–7	
Pipa Americana.		Back of ♀ in the water.	100	
Rhinoderma darwinii.		Gular pouch ♂.	10	
Rhacophorus reticulatus.		On abdomen ♀.	21	
Hyla goeldii.	Not pigmented.	Back of ♀.	10–26	4 mm.
Hylodes martinicensis.	Not pigmented.	Under stones, etc.	30	3–4 mm.
Phyllomedusa jheringii.		On trees.	200	2 mm.
Alytes obstetricans.	Not pigmented.	Round legs of ♂.	18–54	3½–5 mm.
Cystignathus mystaceus.	Not pigmented.	Nest under stones, etc.		
Rhacophorus schlegelii.	Not pigmented.	Nest in the side of a bank.		1 mm.
Polypedates maculatus.	Not pigmented.	Sides of cisterns, etc.		
Paludicola fuscomaculata.	Not pigmented.[10]	Floating in small pool.		1 mm.

[10] A case of unpigmented eggs laid in small pools; these eggs are not long exposed, for they hatch eighteen to twenty-four hours after the beginning of segmentation.

The development of the digestive tract has heretofore scarcely been touched upon in the descriptions of the yolk-laden eggs. We have seen a close similarity in the history of the yolk-mass in Hylodes and in the few forms already studied, and the absence in Hylodes of a coiled intestine.

In the embryo of Hylodes, fed by the yolk in the egg; of Rhinoderma darwinii, developed in the gular sac of the male; of Pipa americana, on the back of the female; of Nototrema oviferum, in the dorsal pouch of the female, no horny teeth or jaws have been found. (The presence or absence of teeth is not recorded in the case of other protected embryos.) It is also to be noted that no adhesive gland is found in Hylodes.

Passing now to the organs of respiration, we find that different parts of the body serve for breathing in different forms, and that there is great diversity in the number and form of the gills, where present.

In the viviparous salamander, the gills are finely feathered, and serve for breathing in the fluid of the uterus. Larvæ put into water do not continue to use their gills unaltered; the gills either undergo certain changes or are lost, and new gills or other organs develop. In Alytes, the eggs are carried for a time on the hind legs of the male, and the external gills are represented by one long branched pair. In Pipa americana, in which the eggs are carried in water by the parent, three pair of external gills develop, but these are lost early and the internal gills appear. In Nototrema oviferum, in which the eggs are carried in the dorsal pouch, one pair of bell-shaped gills occur, which seem to represent two fused gills on each side. They are present before the tadpole leaves the maternal pouch, and also for a short time afterwards. A gill rudiment on the third arch may represent the internal gills, but no others are present. In Nototrema marsupiatum, it is said that there is no trace of external gills in the embryo when it leaves the parent's pouch. Finally, the gills are entirely wanting in Hyla göldii, whose eggs are carried on the back of the female; in Rana opisthodon, whose eggs are found in crevices of rocks; in Rhinoderma darwinii, whose embryos are contained in the gular pouch of the male; and in Hylodes martinicensis, whose eggs develop on land. In Hylodes even the gill-slits are probably wanting.

Respiration is carried on by some other means, which in some of these cases has not been recorded. In Hylodes we have seen that the tail is adapted to this function, i. e., the new function of breathing in air has been assumed by an ancestral larval organ no longer useful in the original way. In Rana opisthodon, the tail is not present in advanced

embryos, and numerous folds on the sides of the embryo probably function as respiratory organs.[11]

In other forms besides Hylodes, the tail differs from the swimming-tail of the tadpole. In Nototrema marsupiatum it is said to be vascular; in the tadpoles of Hyla abbreviatus, which slip about (even before their legs appear) on moist perpendicular rocks, the ventral fin is flattened, and seems to serve as a sucker; in Rhinoderma darwinii the tail is small and weak. In Hypogeophis and in Autodax, the fin is wanting.

Lastly, in Hylodes and in Rana opisthodon the little median horny tip in the upper lip is found in a late embryonic stage, and is used in the latter, at least to rupture the membranes in which the embryo is so long encased.

In conclusion, we find that the Amphibia are a plastic group, and among Anura especially there are patent examples of the adaptation of the larval stages to new external conditions, while the original plan of structure of the adults has remained unchanged. There are also other forms known in which, although the adults are very similar, the embryos have different modes of development. In such cases we must suppose that the embryos have diverged in their development while the end-result of their development, namely, the adult form, has remained virtually unchanged. For example, there are two species of Peripatus very similar in adult stages, but which arrive at the adult form through dissimilar embryonic stages. There are also various species of Alpheus and of Palaemonetes that have very similar adults, but whose embryonic metamorphosis differs according to the degree of abbreviation of the development of the different species.

Furthermore as Wilson has pointed out in this connection, it has been shown in the Ascidians that the same adult form may be produced by methods as different as budding and development from the egg. Finally, it is well established that in certain animals mutilation, or even removal of parts of the egg, does not prevent the development of a normal embryo.

SUMMARY.

Comparing the development of Hylodes with the development of frogs having a tadpole stage, the following points of resemblance and of difference are apparent:

[11] We find not only in embryos but also in adult amphibia, great diversity in breathing; respiration may be carried on by the mouth, the skin and even, according to Ritter and Miller, by vascular toes in Antodax, Plethodon and Brachoceps.

Resemblances.

1. Holoblastic segmentation.
2. The mode of formation of the blastopore, the presence of the yolk plug, and its later withdrawal.
3. The mode of development of the liver, pancreas and lungs.
4. Development of the pronephros (with its three funnels) of the Wolffian duct, and of the mesonephros.
5. The presence of a tail.

Differences.

1. A solid medullary plate.
2. In the alimentary canal:
   (a) Absence of horny jaws and teeth.
   (b) Probable absence of perforated gill-slits.
   (c) Absence of external and internal gills.
   (d) Absence of coiled intestine.
   (e) Extensive closure of the middle region of the archenteron.
3. Simplification of the circulation in the gill region.
4. Early and simultaneous appearance of all four legs.
5. Incomplete development of the lungs and of the pulmonary circulation at the time of hatching as compared with the tadpole when it leaves the water.
6. Forelegs are never completely enclosed by an operculum.
7. Changes in the tail from an organ of locomotion to one of respiration.
8. Presence of a hardened tip on the upper lip.
9. Absence of adhesive glands.

On the assumption that Hylodes has had an ancestor in which a tadpole stage existed, the development has been altered by losses, by additions, and by changes in organs already present. The structures that have been lost are: the horny jaws and teeth, adhesive glands, the perforated gill-slits, the external and internal gills, the coiled intestine, and certain blood-vessels of the gill region and of the lungs. There have been added the hard tip of the upper lip, and the early development of the legs. The tail has changed from a muscular to a vascular organ.

Germantown, Pa., U. S. A., February, 1904.

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EXPLANATION OF PLATES.

The figures are taken from camera drawings. The embryos are magnified in Figs. 1 to 10, inclusive, about thirteen and a half diameters, in Figs. 11 to 14, inclusive, a little more than thirteen and a half diameters, and in Fig. 15 about twenty-eight diameters.

PLATE I.

FIG. 1. Two-celled stage. Upper pole showing the beginning of the second furrow.

FIG. 2. Two-celled stage. Lower pole.

FIG. 3. Later stage of segmentation. Upper pole.

FIG. 4. Same egg as in Fig. 3. Lower pole.

FIG. 5. Stage showing blastopore.

FIG. 6. Stage I. Young embryo with the blastopore almost closed.

FIG. 7. Stage II. Posterior end of the embryo, showing the anus.

FIG. 8. Stage IV. Head end.

FIG. 9. Same embryo as in Fig. 8. Tail end.

FIG. 10. Stage VI.

PLATE II.

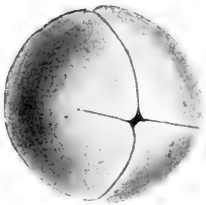
FIG. 11. Stage IX, as seen from the side.

FIG. 12. Stage XII.

FIG. 13. Stage XIII.

FIG. 14. Stage XIV.

FIG. 15. Stage VIII. Anterior end of the embryo, as seen from the ventral side, when cut off the yolk. Y, point where the yolk has been cut away from the gut.



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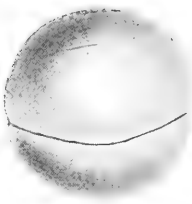

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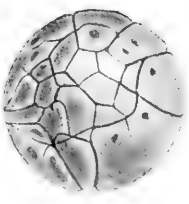

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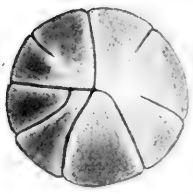

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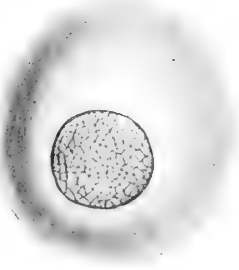

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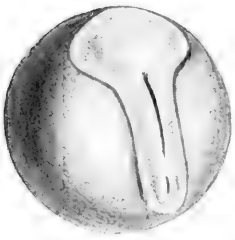

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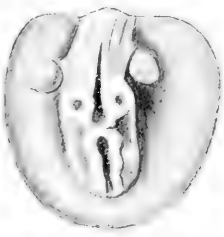

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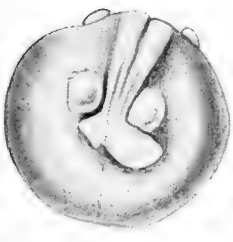

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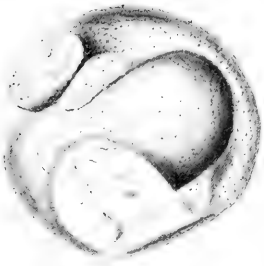

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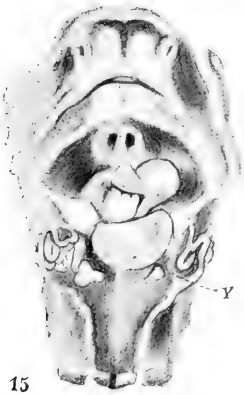

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13

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE EYE IN AMPHIBIA.

I. On the Origin of the Lens. *Rana palustris.*

BY

WARREN HARMON LEWIS, M. D.

From the Anatomical Laboratory of Johns Hopkins University.

With 42 Text Figures.

This article is intended as a contribution to the study of *correlative embryology, i. e.,* the influence of intra-organic environment in development. Our problem is to determine how far the various organs and tissues of an organism are dependent or independent of the various other organs and tissues for their origin, differentiation and growth. An organ may be dependent or independent (self differentiating) of other organs for its initial origin, or for its subsequent development, or it may be dependent on a series of influences for its proper development.

It will be natural to inquire into the nature of these influences to determine if they are mechanical, chemical, electrical or unknown influences still to be discovered.

In the present paper I shall show:

A. That the lens does not arise from the ectoderm without the contact influence of the optic vesicle.

B. That the optic vesicle can stimulate a lens to form from various portions of the ectoderm and even from ectoderm from the abdomen of another species of frog, indicating thereby that there is no especial predetermined area of the ectoderm, which must be stimulated in order that a lens may arise.

C. That various portions of the optic vesicle have the power of stimulating lens-formation.

Spemann[1] has shown indirectly that the lens is dependent on the influence of the optic vesicle for its origin from the epithelium of the skin. He employed the method of destroying partially or entirely the rudi-

[1] Ueber Correlationen in der Entwickelung des Auges. Verhandl. der Anat. Gesell., 1901.

ment of the optic vesicle by a hot needle or an electric cauterizing needle. This was done on the early stages of triton embryos before the closure of the neural canal. At this time the beginnings of the optic vesicles can be seen at the anterior end of the medullary plate. Their destruction usually involves more or less injury to the neighboring parts, especially the brain, but need not interfere with that portion of the epithelium which normally gives rise to the lens. Spemann's experiments show that in some instances the rudiment of the optic vesicle was so far destroyed that its subsequent regeneration and growth were insufficient to bring it into contact with the skin epithelium. In all such cases where the optic vesicle remained deep, there was absence of lens formation on that side. If, however, the injury to the optic vesicle rudiment was not sufficient to prevent the more or less regenerated optic vesicle from touching the skin epithelium, a lens was formed at the point of contact between the optic vesicle and ectoderm.

Herbst[2] likewise concludes from a study of normal and abnormal specimens that the contact of the optic vesicle on the ectoderm is necessary for lens formation.

METHODS AND MATERIAL.

In order to test these and other questions, I employed during the spring of 1903 a quite different method from Spemann's, one which I believe is capable of extension to many other organs and tissues. One can, with the aid of a binocular microscope and very delicate instruments, make exceedingly minute dissections of the living amphibian embryo and can remove various organs, transplant them, or alter the normal relations and so alter the influences they exert on each other. We may thus determine certain correlations necessary to normal development. Besides this modification of Born's[3] method I have found Harrison's[4] method of grafting different-colored species of frog embryos together (heteroplastic grafting) very useful.

The embryos of Rana palustris and R. sylvatica were used for the experiments. Serial sections 10 micro mm. in thickness were made of the embryo in each experiment. They were stained in hæmatoxylin and Congo red.

[2] Formative Reize in der Tierischen Ontogonese, 1901, p. 60.

[3] Born, G. Ueber Verwachsungsversuche mit Amphibienlarven. Archiv f. Entwickelungsmechanik, Bd. IV, 1896-97.

[4] Harrison, R. G. The growth and regeneration of the tail of the frog larva. Archiv f. Entwickelungsmechanik, Bd. VII.—Experimentelle Untersuchungen ueber die Entwickelung der Sinnesorgane der Seitenlinie bei den Amphibien. Archiv f. mik. Anat. u. Entwickelung., Bd. 63, 1903.

The operations were performed on the right side and the uninjured left side used for comparison. All the figures were made with the aid of a camera lucida.

A. Will the Lens Arise from the Ectoderm without the Contact of the Optic Vesicle on the Skin?

Series DF and IV.

In the first series[5] of experiments, series DF and IV, the optic vesicle was removed at a stage before it had had any visible influence on the skin leading to lens formation, and transplanted some distance caudally. The experiments were performed on the embryos of Rana palustris shortly

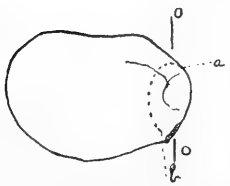

Fig. 1.

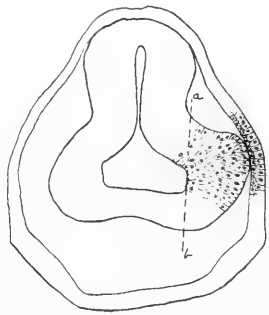

Fig. 2.

Fig. 1. Outline of stage II. ×8 diameters. The dotted line *ab*, incision for skin flap. *oo*, plane of section of Fig. 2.

Fig. 2. Outline of section through the head of stage II in the plane, *oo* Fig. 1. *ab*, line along which optic vesicle was cut away. ×41 diameters.

after the closure of the medullary folds, see Figs. 1 and 2. An incision was made along the line *ab,* see Fig. 1, and the anterior skin flap carefully turned forward without tearing either it or the structures beneath. It is quite easy at this stage, as the optic vesicle is not adherent to the skin. At a later stage, however, the optic vesicle becomes quite firmly adherent to the skin and can scarcely be separated from it without tearing either one or the other, and consequently is unsuitable for the operations of series DF or IV. After the skin flap is laid forward and the small round optic vesicle exposed, the latter was cut off close to its attachment to the brain, leaving a large opening into the brain

[5] All the experiments of a series were done on embryos of an age as near alike as possible and as near as possible in the same manner. A certain amount of variation in the experiments of a series was unavoidable and consequently the results vary somewhat.

cavity. The detached optic vesicle was transplanted by pushing it caudally beneath the skin into the mesenchyme from the incision *ab,* Fig. 1 and allowed to develop there. The optic stalk is not formed at this stage, as will be seen in Fig. 2. The optic vesicle and its cavity are more or less conical in shape with the base attached to the brain. The skin flap after the removal of the optic vesicle is turned back to its original position and held there by the pressure of small pieces of silver wire for a few minutes. Healing is rapid and a few hours are generally sufficient to close the wound along the line of incision *ab,* Fig. 1.

Experiment DF_{54}.

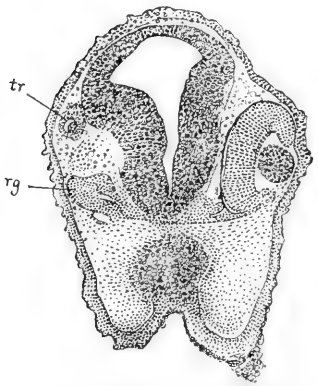

FIG. 3. Experiment DF_{54}. Transverse section through the middle of the regenerated eye. × 45 diameters. *rg*, regenerated eye. *tr*, cephalic end of transplanted eye.

The embryo, experimented upon as described above, was killed two days after the operation. The sections show a well-formed optic cup on the left side with a lens about 70μ in diameter. This lens is entirely separate from the ectoderm, see Fig. 3. On the right side is a small regenerated eye separated superficially from the ectoderm by a single layer of mesenchymal cells. The ectoderm is continuous over the region of this regenerated eye and shows no traces of changes leading to lens-formation. If the conditions had remained normal a lens of the diameter of the normal one would have been present, or as we shall see later if the regenerated eye were in contact with the skin changes leading to lens-formation such as are found in experiments DF_{55} and DF_{56} would probably be present. The transplanted portion of this eye lies caudo-dorsally to the regenerated eye and is quite superficial. It is about as large as the normal eye on the left side, has evaginated but shows no signs of lens-formation. I think it very probable that the regenerated eye would soon have stimulated lens-formation as in experiments DF_{53}, DF_{55} and DF_{56}.

Experiments DF_{53}, DF_{55}, *and* DF_{56}.

The embryos of these three experiments were killed as in DF_{54} two days after the operation. In DF_{53} and DF_{55} the regenerated optic vesicle is in contact with the ectoderm at which point the inner layer of the skin is thickened showing the first beginnings of lens-formation,

see Fig. 4. In experiment DF_{56} there is over the regenerated eye a large lens about 50μ in diameter but still quite broadly adherent to the ectoderm, see Fig. 5. The normal lens of the left side is about 70μ in diameter and shows considerable advance in differentiation.

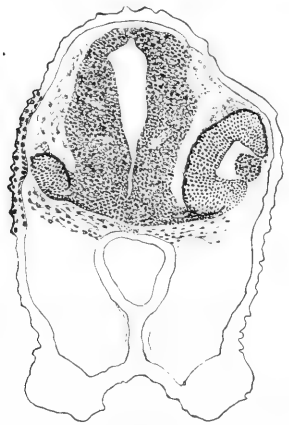

FIG. 4.

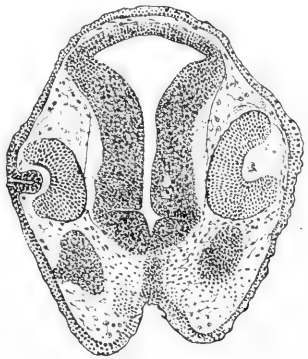

FIG. 5.

FIG. 4. Experiment DF_{55}. Transverse section through the middle of the regenerated eye. × 45 diameters.

FIG. 5. Experiment DF_{56}. Transverse section through the middle of the regenerated eye. × 45 diameters.

Of four experiments of the series DF in which the embryos were killed two and one-quarter days after the operation, the eyes have regenerated sufficiently in each to reach the ectoderm and in each instance there is a lens, still attached to the skin in three and detached in the fourth.

In sixteen embryos of the series DF, killed three days after the operation, fourteen showed regenerated eyes of various sizes. Of these, eleven touch, or nearly touch the skin and ten of them have lenses and three of the nine are still attached to the ectoderm. Three of the regenerated eyes are small and separated from the ectoderm by a considerable layer of mesenchyme and in these the skin shows no sign of lens-formation. In two of the sixteen experiments no traces of regenerated eyes were present and there were no signs of lens-formation or of changes in the ectoderm indicating where the lens would have arisen under normal conditions with the eye present.

Of twenty-two embryos of the series DF, killed four days after the operation, twenty-one show regenerated eyes of various sizes. Of these regenerated eyes nine touch or nearly touch the skin and have lenses, three of which are still attached to the ectoderm. Seven of the regene-

rated eyes are separated from the skin by varying amounts of mesenchyme, they have lenses, three of which are still attached to the ectoderm, see Figs. 16, 21, 23 and 25. Five of the regenerated eyes are deeply buried in the mesenchyme and probably were never at any time in contact with the skin, see Fig. 17. Of these five, none have lenses nor show any signs of lens-formation either in the ectoderm or from the optic cup itself. The one experiment in which no signs of a regenerated eye occurred, shows no signs of lens-formation in the ectoderm which would have given rise to a lens had the eye remained in its normal position.

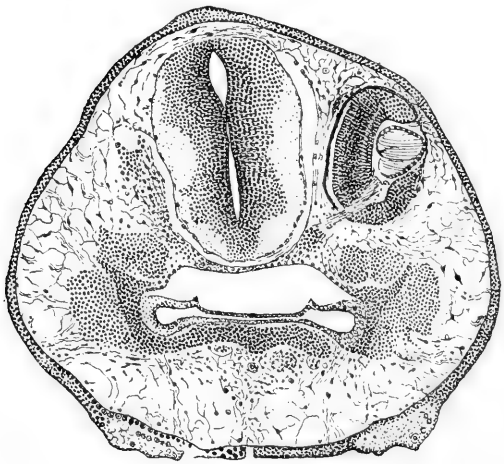

FIG. 6.

FIG. 7.

FIG. 6. Experiment IV_1. Transverse section through the middle of the eye region. × 60 diameters.

FIG. 7. Experiment IV_1. Outline of tadpole 5 days after the operation. The right transplanted eye is seen in black. × 4 diameters.

Of three embryos, killed four and one-half days after the operation, in one the regenerated eye is near the ectoderm and has a large lens, two are deeply buried in the mesenchyme and are without lenses.

Experiment IV_1.

This embryo experimented on as above was killed five days after the operation. There is no trace of an eye in the region from which the optic vesicle was removed. Mesenchyme has filled in the space which would normally have been occupied by the optic cup and lens so that there is very little difference in the size of the two sides of the head (see Fig. 6).

On the left or normal side is a large, well-formed optic cup and lens, but on the right side there is not a trace of a lens or of a change in the epithelial cells from which the lens would have arisen had the conditions remained normal. The transplanted optic vesicle had been pushed somewhat deeply beneath the skin into the cephalic end of the Wolffian body caudal to the optic capsule (see Fig. 7). An examination of the section shows that some of the brain adjoining the optic vesicle was cut off and transplanted with it. There is no trace of a lens near this transplanted optic vesicle.

Of twenty-one embryos of the series DF and IV_1, killed five days after the operation, fourteen have regenerated eyes and seven no traces of regenerated eyes or lenses. Of the fourteen regenerated eyes seven are more or less superficial and have lenses, the remaining seven are deeply buried in the mesenchyme and of these six show no traces of lenses. One of the deeply situated regenerated eyes is quite small but has a lens which is still attached by a long narrow pedicle to the inner layer of the ectoderm (see Fig. 8). The sections are cut obliquely to this pedicle and in Fig. 8 it is drawn from a flat reconstruction of the several sections through which it passes.

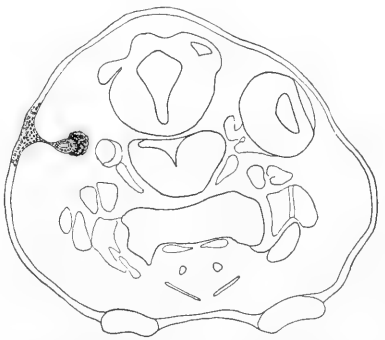

FIG. 8. Experiment IV_5. Section through the regenerated eye. × 33 diameters.

One embryo, killed five and one-half days after the operation, shows a deeply placed regenerated eye without trace of a lens.

Of eleven experiments in which the embryos were killed seven days after the operation, ten show regenerated eyes with lenses while one lacks the regenerated eye and lens entirely.

Three embryos, killed eight days after the operation, show large regenerated eyes with lenses.

Two embryos were killed nine days after the operation, both showing regenerated eyes, superficially situated, but in one the lens is absent.

Two embryos were killed eleven days after the operation, one shows a regenerated eye with a lens, the other, experiment IV_3, lacks entirely an eye or lens in the normal place on the right side.

Experiment IV_3.

The embryo of R. palustris was experimented on as described above and killed 11 days after the operation. There is no sign of an eye or

lens in the normal place on the right or operated-on side. The skin over this region shows no signs of lens formation. Mesenchyme has partially taken the place of the eye but not entirely, and there is a depression upon this side of the head (see Fig. 9). The transplanted optic vesicle lies in the cephalic end of the Wolffian body, deep beneath the skin. It has continued enlarging, invaginating and differentiating, but without signs of a lens.

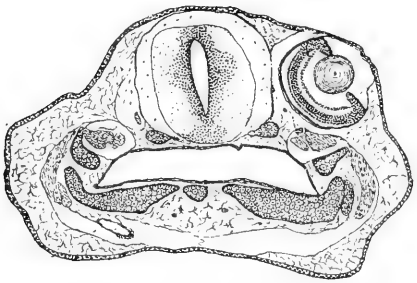

FIG. 9. Experiment IV_3. Transverse section through the middle of the eye region. × 30 diameters.

In series DF and IV there were ninety experiments. The embryos were killed from two to eleven days after the operations. In fifty-seven of the embryos there was regeneration of the eye and lens-formation. In several of these the regenerated eye was more or less deeply situated but the instances where the lens is still attached to the ectoderm by a long narrow pedicle give a clue to the origin of the lenses in the other experiments where the regenerated eye and lenses are not close to the skin. In three of the experiments where the eyes were more or less superficial, no traces of lens-formation are to be found. In seventeen experiments the regenerated eyes were deeply situated without possibility of direct contact with the ectoderm and in no instance was there a trace of lens-formation. In thirteen experiments regeneration of the eye failed and in each instance no traces of lens-formation were found.

That the operation of turning the skin flap forward and then replacing after removal of the optic vesicle does not interfere with lens formation is evident from the numerous instances in which the regenerated eye has enlarged sufficiently to touch the skin and stimulate lens formation, see Figs. 4, 5, 8, 14, 21, 23 and 25.

Experiment XI_{72}.

This experiment, performed in a quite different manner from those above, suggests the view that the lens will not form without the contact influence of the optic vesicle on the skin. The skin over and about the optic vesicle of R. palustris of stage II (see Figs. 1 and 2) was completely torn away and the head one-half of a slightly older embryo of R. sylvatica was grafted upon the denuded area by its cut end. Nine

days after the operation the tadpole was killed. Fig. 10 shows a condition which did not occur in any others of this series. The œdema which commonly occurs in the grafted-on pieces of R. sylvatica seems to have spread to the R. palustris, and has given rise to the thick layer of mesenchyme between the left eye and the skin. This layer of mesenchyme has prevented the optic vesicle from touching the skin, and as a result the lens has not formed on this side as it did in all the other operations of this series. The right eye, over which the graft was placed, projects towards the cœlom of the R. sylvatica. A lens is wanting on this side also, owing, I think, to the lack of contact between optic vesicle and skin.

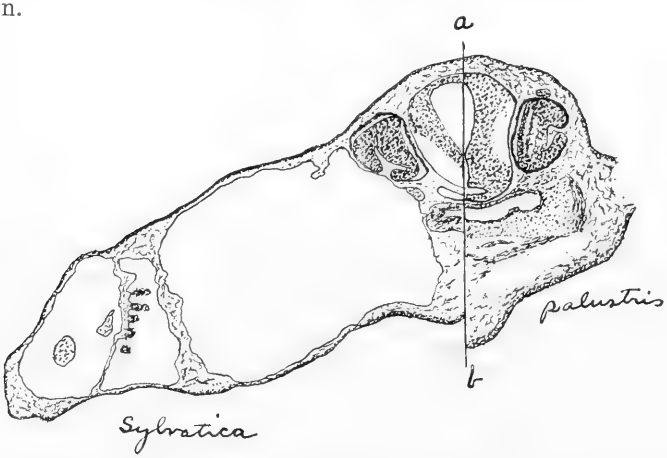

FIG. 10. Experiment XI_{72}. Section through the eye region. The two portions, one on either side of the line *ab* are drawn from slightly different levels in order to have the section pass through the middle of each optic cup. R. palustris on the right and R. sylvatica on the left. × 30 diameters.

CONCLUSION.

It is evident then that the epithelial cells which normally give rise to a lens do not do so when the optic vesicle fails to come in contact with them for a sufficient length of time or at the proper time. On the other hand, when the optic vesicle, entire or regenerated, comes in contact at the proper stage of development with the skin on the side of the head a lens generally is formed. So we must conclude, I think, that during normal development the lens is dependent for its origin upon the influence exerted by the optic vesicle upon the skin at the side of the head. This conclusion receives additional support from the experiments of section B of this paper in which the optic vesicle stimulated lens formation from skin which under normal conditions never gives rise to a lens.

B. Can the optic vesicle stimulate lens-formation from ectoderm other than that which normally gives rise to the lens?

Is there a Special Predetermined Area of the Skin which must be Stimulated by the Optic Vesicle in order that a Lens may arise?

Experiment DF_{56}.

Two days after the operation the embryo was killed. The sections show a fair sized regenerated eye with a lens still broadly adherent to the inner layer of the ectoderm and continuous with it, see Fig. 5. This lens is about 50μ in diameter while the normal one on the uninjured left side is about 70μ in diameter, and completely separated from the

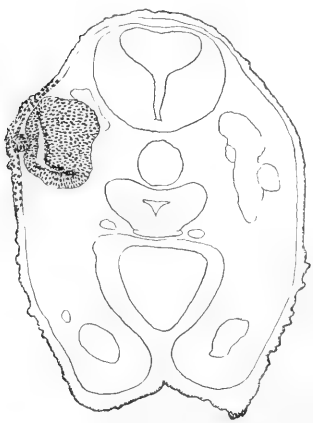

Fig 11.

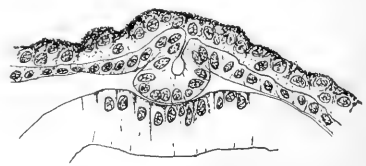

Fig. 12.

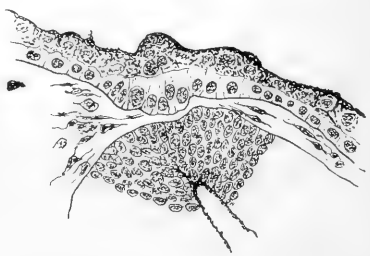

Fig. 13.

Fig. 11. Experiment DF_{56}. Transverse section through the transplanted eye. × 45 diameters.

Fig. 12. Experiment DF_{56}. Section through the lens of the transplanted eye. × 200 diameters.

Fig. 13. Experiment DF_{50}. Section through the transplanted eye showing an early stage of lens-formation. × 200 diameters.

ectoderm. Figure 11 through the transplanted eye, is from a section 130μ caudal to the section indicated in Fig. 5. Here the large irregular transplanted eye lies close to the ectoderm, the inner layer of which is thickened and bent into a lens-like process about 40μ in diameter. The cells of this lens are continuous with those of the inner layer as will be seen in Fig. 12. This experiment alone is sufficient to demonstrate that the optic vesicle can stimulate lens-formation from strange epithelium and that there is no predetermined area of the skin which must be stimulated in order that a lens may arise.

Two other embryos of the series DF, killed two days after the operation, show thickenings of the inner layer of the ectoderm over the regenerated eyes (see Fig. 4) and also thickenings over the transplanted eyes. These beginnings of lens-formation are in neither experiment so far advanced as found in experiment DF_{56}. In another experiment, DF_{54}, Fig. 3, the transplanted eye nearly touches the skin but no trace of of lens formation is present.

Experiment DF_{50}.

The embryo was killed two and one-quarter days after the operation. The regenerated eye touches the ectoderm at which point there is a thickening of the inner layer indicating the beginnings of lens-formation. The transplanted eye, which is quite as large as the normal one but irregular in form, lies between the regenerated eye and the otic capsule.

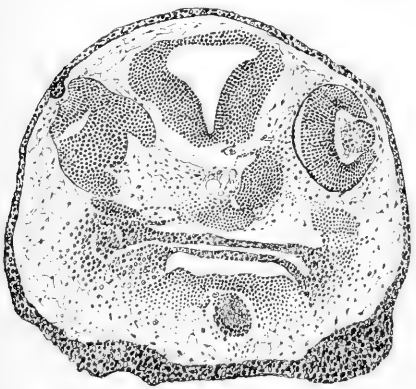

FIG. 14.

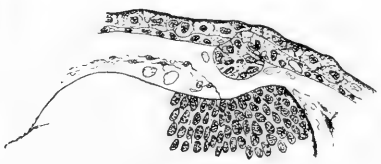

FIG. 15.

FIG. 14. Experiment DF_{11}. Oblique section through the transplanted eye. × 45 diameters.
FIG. 15. Experiment DF_{11}. Section through the lens and edge of transplanted eye. × 175 diameters.

At the point where it touches the ectoderm there is a thickening of the inner layer, the beginning of lens formation, see Fig. 13. Two other embryos killed at this period show the transplanted eye close to the skin, but lens formation is wanting.

Of sixteen embryos in the series DF, killed three days after the operation, twelve of the transplanted eyes are superficial and of these four show lenses, three are still attached to the ectoderm. Three of these four embryos with lenses over the transplanted eyes also show lenses over the regenerated eyes, the fourth regenerated eye had not reached the ectederm and is without a lens. In four of the sixteen experiments the transplanted eyes are deeply situated in the mesenchyme. One and only one of these possesses a lens and as this lens is still attached to

the ectoderm by a long narrow pedicle there can be but little doubt as to its origin. This long epithelial process was probably formed at the time of the healing of the operation wound and thus gave the deeply placed eye a chance for contact with the ectoderm.

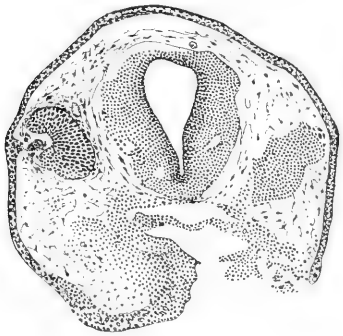

FIG. 16.

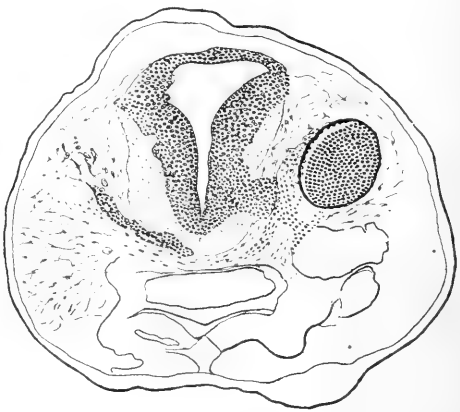

FIG. 17.

FIG. 18.

FIG. 19.

FIG. 16. Experiment DF_{11}. Oblique section through the regenerated eye. × 45 diameters.
FIG. 17. Experiment DF_{47}. Oblique section through the regenerated eye. × 45 diameters.
FIG. 18. Experiment DF_{47}. Section through lens and edge of transplanted eye. × 175 diameters.
FIG. 19. Experiment DF_{47}. Section through transplanted eye. × 45 diameters.

Experiment DF_{11}.

The embryo was killed four days after the operation. Fig. 16 from a section through the regenerated eye shows a lens about 40μ in diameter that has apparently just broken away from its attachment to the inner layer of the ectoderm. Fig. 14 is from a section 270μ caudal to

the section in Fig. 16. The irregular transplanted eye lies between the otic capsule and the regenerated eye. The transplanted eye is quite superficial and at the place where it touches the ectoderm there is developing a small lens, about 45μ in diameter which is still broadly adherent to and continuous with the inner layer of the ectoderm. See Fig. 15. The section seen in Fig. 14 is cut obliquely and shows on the opposite side the normal left eye with a lens about 135μ in diameter. Here as in experiment DF_{56} two lenses are developing on the same side of the head, one in the normal position for the regenerated eye and the other some distance caudal to this for the transplanted eye.

Experiment DF_{47}.

As in the preceding, the embryo was killed four days after the operation. The regenerated eye, deeply situated in the mesenchyme, consists of an optic stalk with a small knob of cells at the end, see Fig. 17. There is no sign of a lens in its neighborhood. The transplanted eye is large and superficially situated between this regenerated eye and the otic capsule. It has two lenses. One of them is quite small, 30μ in diameter and still attached to the inner layer of the ectoderm, see Fig. 18. Fig. 19, from a section 100μ cephalad to the section of Fig. 18, shows the second lens and a portion of the same eye shown in Fig. 18. This lens is about 60μ in diameter and is separated from the skin by a layer of mesenchyme. It is most probable that this lens came from the overlying ectoderm, as it shows some differentiation. There are no signs of its having arisen from the optic cup and the lens is farther from the edge of the optic cup than from the skin. Experiments DF_{43}, see Fig. 23, and DF_{44}, see Fig. 25, illustrate clearly how such a lens might have arisen.

Experiment DF_{13}.

The embryo was killed four days after the operation. The regenerated eye is small and deeply situated, much as in experiment DF_{47}. It shows no traces of a lens in its neighborhood or in connection with the overlying skin. The transplanted eye is large and quite regularly invaginated, see Fig. 20. It lies between the regenerated eye and the otic capsule. Lying between the outer layer of the optic cup and the skin is a small lens about 30μ in diameter. From its position and the condition of the overlying ectoderm it seems likely that it has been recently derived from the ectoderm. If it had been derived from the optic cup we should hardly expect it to lie in this position. A slight irregularity on the superficial surface of the lens, a small projection from the over-

lying ectoderm and modification of the inner layer indicate the ectodermal origin of the lens.

Experiment DF_{45}.

The embryo was killed four days after the operation. The regenerated right eye is of medium size and is separated from the ectoderm by a layer of mesenchyme, see Fig. 21. The lens which is about 70μ in diameter lies in the optic cup and has apparently just separated from the inner layer of the ectoderm from which there is a small irregular projection corresponding to an irregularity on the superficial surface of the lens. The transplanted eye which lies just caudal to the regenerated eye is as large as the normal one on the left side. It is separated from

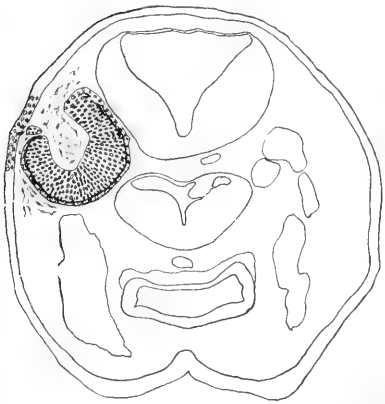

FIG. 20.

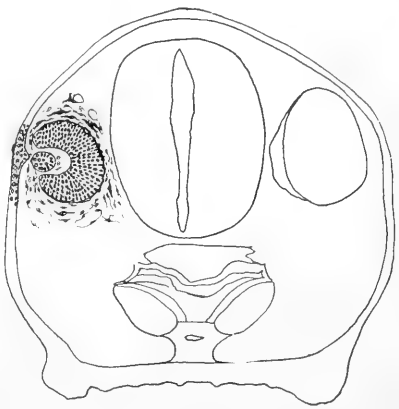

FIG. 21.

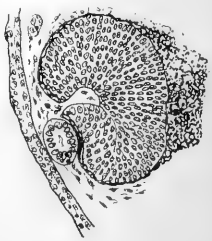

FIG. 22.

FIG. 20. Experiment DF_{13}. Section through transplanted eye and lens. × 50 diameters.
FIG. 21. Experiment DF_{45}. Section through regenerated eye and lens. × 50 diameters.
FIG. 22. Experiment DF_{45}. Section through transplanted eye and lens. × 100 diameters.

the ectoderm by a thin layer of mesenchyme. Fig. 22 is from a section through the cephalic end of the eye and shows between the outer layer of the optic cup and the ectoderm a lens about 30μ in diameter. It is impossible to determine the exact origin of this lens but the preceding and succeeding experiments clearly indicate that it is of ectodermal origin.

Experiment DF_{43}.

The embryo was killed four days after the operation. The sections show a rather deeply seated medium sized regenerated eye, see Fig. 23. The small lens is still attached to the inner layer of the ectoderm by a long narrow pedicle which extends through the rather thick layer of mesenchyme separating the optic cup and skin. Had the embryo been killed

a day or so later all traces of this pedicle might have been lost and it would have been impossible to determine the origin of the deeply seated lens. The transplanted eye which is nearly as large as the normal eye

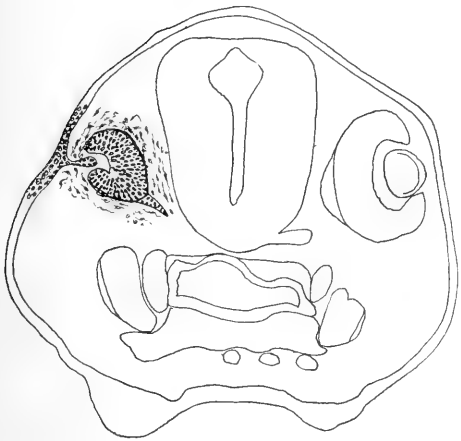

FIG. 23.

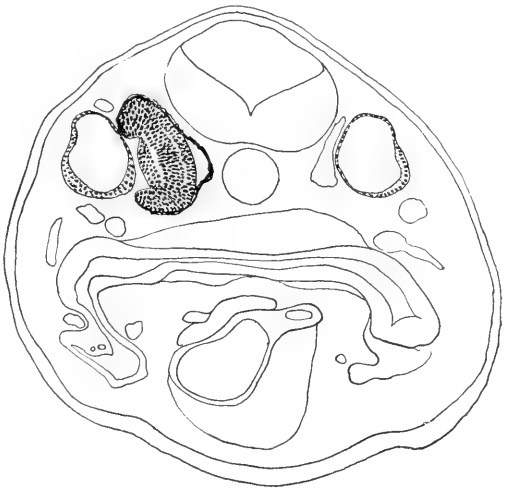

FIG. 24.

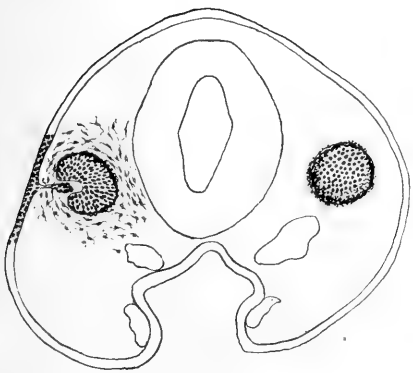

FIG. 25.

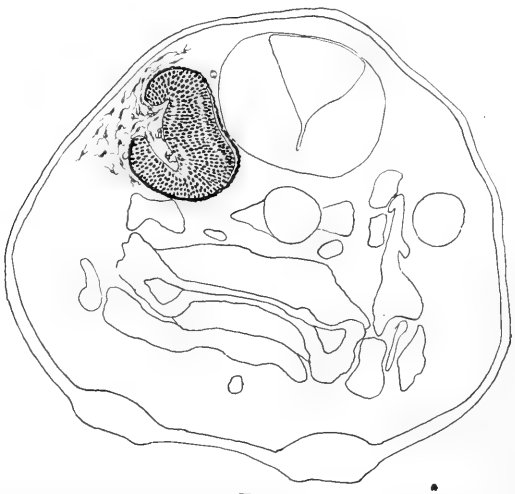

FIG. 26.

FIG. 23. Experiment DF_{43}. Transverse section through regenerated eye and lens. × 50 diameters.

FIG. 24. Experiment DF_{42}. Transverse section through transplanted eye and otic capsules. × 50 diameters.

FIG. 25. Experiment DF_{44}. Oblique section through regenerated eye and lens. × 45 diameters.

FIG. 26. Experiment DF_{44}. Section through transplanted eye. × 50 diameters.

on the left side, is invaginated and shows differentiation of the layers of the retina. It is deeply situated being on the mesial side of the otic capsule, see Fig. 24. There is no sign of a lens in connection with it.

Experiment DF_{44}.

As in the experiment above the embryo was killed four days after the operation. The condition of the regenerated eye is very similar to that in experiment DF_{43}. The medium sized regenerated eye is separated from the ectoderm by a considerable thickness of mesenchyme. A small lens about 40μ in diameter lies in the optic cup. It is still connected with the ectoderm by a long narrow epithelial pedicle which passes through the layer of mesenchyme separating the optic cup and skin. The sections of this embryo are cut obliquely to the stalk and in Fig. 25 the stalk is from a composite of two sections. The transplanted eye is situated just in front of the otic capsule and between it and the skin is a considerable thickness of mesenchyme, see Fig. 26. The transplanted eye is about as large as the normal one on the left side. It shows invagination and differentiation of the layers of the retina. There is no trace of a lens in its neighborhood. The diameter of the normal lens is about 140μ.

The conditions found in the regenerated eyes in the above two experiments can readily be explained on the supposition that the pressure of the silver wire on the skin flap over the stump of the amputated eye was sufficient to hold the ectoderm in contact with the regenerating eye until the adhesion between them was accomplished. This adhesion which is normally followed by the development of the lens had the same effect in these experiments, but during the process of lens-development the rapidly growing mesenchyme forced its way between the eye and skin pushing the latter outward and separating it from the small eye and its short optic stalk. A pull was thus exerted on the inner layer of the ectoderm owing to the adhesion between the lens and eye.

Three other embryos killed four days after the operation have superficially situated transplanted eyes with lenses. Eleven embryos killed at the same time show deeply situated transplanted eyes without traces of lens-formation or lenses in their neighborhood.

Of three embryos, killed four and one-half days after the operation, in two the regenerated eyes were deeply situated and without lenses. The transplanted eyes in these two were superficial, one has a lens and the other is without one. In the third experiment the regenerated eye is superficial and has a lens while the transplanted eye is deeply placed and is without a lens.

Experiment IV$_9$.

The experiments of this series (IV), as we have already noted, were performed on Rana palustris at a stage before there is a trace of lens formation and before the preliminary adhesion between optic vesicle and skin takes place (see Figs. 1 and 2). In this experiment the right optic vesicle and some adjoining brain tissue were transplanted by being pushed underneath the skin from the incision *ab* (see Fig. 1). Five days after the operation the tadpole was killed. Fig. 27 shows an outline of the tadpole when in xylol. The irregularly shaped black eye is some distance caudal to the normal position. In the normal position there is a slight depression but no trace of an eye or lens. The sections show that one corner of the transplanted eye lies near the skin in the region of the otic capsule. The invagination is quite irregular, as

Fig. 27.

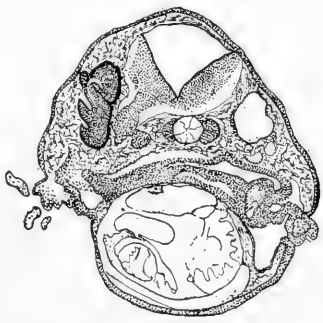

Fig. 28.

Fig. 27. Experiment IV$_9$. Outline of tadpole five days after the operation. Drawn in xylol. The transplanted eye is seen in black. × 4 diameters.

Fig. 28. Experiment IV$_9$. Section through the plane *oo*, see Fig. 27. The irregular transplanted optic cup and the small lens are seen. × 30 diameters.

will be seen in Fig. 28. A small lens 60 micro mm. in diameter and still attached to the skin by a narrow stalk lies between this superficial portion of the optic cup and the skin. The normal lens on the left side is about 130 micro mm. in diameter and farther differentiated than the right one. The sections show no trace of a regenerated eye in the normal position on the right side nor are any changes in the ectoderm indicating lens formation. A section through this region is very much like that seen in Fig. 6.

Of twenty-one embryos, killed five days after the operation, the transplanted eyes are superficial in ten and eight of these have lenses. Of the eleven deeply situated transplanted eyes but one has a lens and it is still connected with the ectoderm by a long narrow pedicle. In two of the above experiments both the regenerated and transplanted eyes of the same embryo have lenses.

Experiment DF_{33}.

The embryo was killed seven days after the operation. There is no trace of a regenerated eye or lens in its neighborhood. The large and very irregular transplanted eye lies ventral to the otic capsule. The eye must have been injured gratly in transplanting and although differentiation of the retina has gone on the various layers are very much mixed up. This eye is very deeply situated and has two large and well-formed lenses, each at about the same stage of differentiation as the normal one on the left side. The two lenses are some distance apart, one projecting from the cephalic surface of the eye and lying ventro-mesially to the otic capsule, it measures about 140μ in diameter; the other projects caudally and lies dorsal to the anterior end of the Wolffian body, it is about 100μ in diameter. The normal lens on the left side is about 180μ in diameter. Neither of these lenses could have come from the overlying ectoderm, they must have originated either from the optic cup itself or from epithelial cells carried from the edge of the wound into the present situation with the transplanting of the eye. That the extensive injury to the optic vesicle may have stimulated it in some way to give rise to the lenses is not improbable.

In three other embryos, killed seven days after the operation, the transplanted eyes are deeply buried and without lenses.

Two embryos, killed eight days after the operation, have deeply situated transplanted eyes without lenses.

Of two embryos, killed eleven days after the operation, one lacks entirely a regenerated eye and lens and the transplanted eye is deeply situated and without a lens. The other shows a regenerated eye with a lens and a superficially transplanted eye without a lens.

There are seventy-one experiments of the series DF and IV in which the embryos show transplanted eyes. The embryos as we have already noted were killed from two to eleven days after the operation. Thirty-eight embryos show superficially situated transplanted eyes, that touch or nearly touch the ectoderm, some of them are separated from the ectoderm by a thin layer of mesenchyme. Twenty-five of these have lenses (one eye has two lenses), nine of the lenses are still attached to the ectoderm; seventeen of the lenses are separate, but lie in such a position as to indicate that they might have come from the ectoderm. In thirteen embryos the more or less superficially situated transplanted eyes touch or nearly touch the ectoderm and are without lenses. Why the optic vesicles should not have stimulated lens-formation in these experiments cannot be determined at present. In thirty embryos the transplanted

eyes are deeply situated and are without lenses. In two deeply situated transplanted eyes lenses are present but still attached to the ectoderm by long pedicles. In the other embryo, however, the deeply situated transplanted eye has two lenses neither of which could have come from the overlying ectoderm. With the exception of this last instance there is no indication of the origin of a lens from the edge of the optic cup.

Series XI.

In the experiments of series XI the skin over the right side of the head was completely torn away so as to expose the optic vesicle and structures immediately about it. This was done on Rana palustris at the same stage as experiments of series DF and IV, see Figs. 1 and 2. The anterior half or more of a slightly older embryo of Rana sylvatica was grafted by the cut surface onto this denuded area of R. palustris.

Experiment XI_{75}.

In experiment XI_{75} the head half of a Rana sylvatica embryo was grafted onto the right side of the head of a younger Rana palustris. The graft was placed with the caudal cut surface against the denuded area and held in place for about an hour by small pieces of silver wire, after which time fusion was fairly well established. The optic vesicle at the time of and shortly after the grafting projected towards the yolk and intestine of Rana sylvatica. After a few days, however, the right eye of R. palustris became visible beneath the skin on the ventral side of R. sylvatica (see Fig. 29). Nine days after the operation the embryo was killed. One portion of the irregularly invaginated optic cup is superficial and not far beneath the skin. Between it and the skin is a small, fairly well-formed lens, about 90 micro mm. in diameter, while the normal lens on the left side is about 150 micro mm. in diameter. It is impossible to determine if the lens of the transplanted eye has arisen from the skin of R. sylvatica or of R. palustris, but in either case it could not have come from the skin originally destined to give rise to it, as that was completely torn away at a period before there is any trace

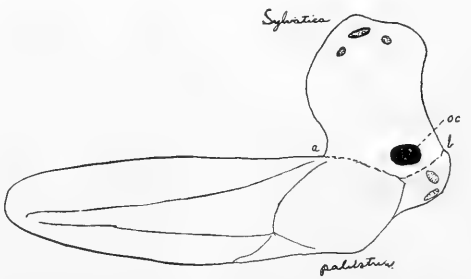

FIG. 29. Experiment XI_{75}. Outline 9 days after operation. *ab*, line of junction between R. sylvatica and palustris. *oc*, eye. × 4 diameters.

of lens formation or even before the adhesion of the optic vesicle and skin. This adhesion takes place before there are any visible signs of epithelial differentiation for the lens. In nine other similarly performed experiments of this series the eye remained deeply buried for periods of 5 to 12 days after the grafting without sign of lens formation.

In the three following experiments of series XI, performed in the same manner as experiment XI_{75}, one corner of the optic vesicle came in contact with the skin and a lens has developed between the optic vesicle and the skin at this place.

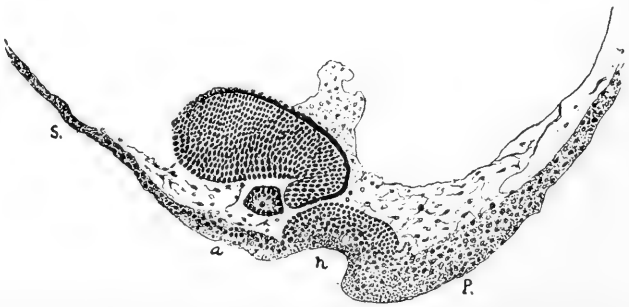

FIG. 30. Experiment XI_{32}. Section through right optic cup and lens. *s*, sylvatica. *p*, palustris, *a*, region of the overlapping of the skin of Rana sylvatica by that of Rana palustris. *n*, nasal pit. × 75 diameters.

Experiment XI_{32}.

The embryo of experiment XI_{32} was killed 5 days after the operation. One corner of the optic cup has reached the surface near the nasal pit at about the junction of the skin of R. palustris with that of R. sylvatica (see Fig. 30). A small lens about 70 micro mm. in diameter lies between the optic cup and skin and probably has arisen from the skin of R. palustris close to the nasal pit. The normal lens on the left side measures about 100 micro mm. in diameter.

Experiment XI_{30}.

As in experiments XI_{75} and XI_{32} the head end of a R. sylvatica embryo was grafted onto the right side of the head of a R. palustris from which the skin had been carefully torn off. At first the optic vesicle was deep beneath the graft and could be seen projecting into the distended cœlom of the R. sylvatica, see Fig. 31. Eight days after the operation the tadpole was killed. One corner of the somewhat irregular optic cup was found to approach near the skin at about the junction of the abdomen of R. sylvatica and R. palustris and near to the nasal pit

of the latter. Here between this corner of the optic cup and a small skin depression is a lens about 90 micro mm. in diameter, while the normal left lens is 160 micro mm. in diameter. The abnormally placed lens on the right side is not only smaller but is less differentiated than the left one.

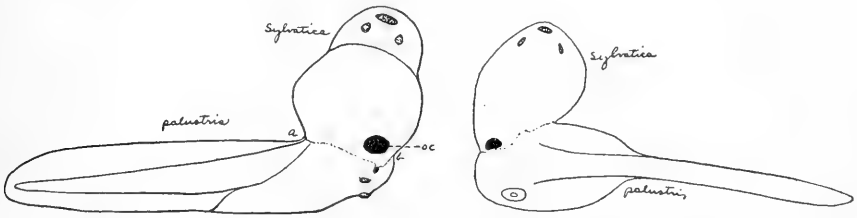

FIG. 31. FIG. 32.

FIG. 31. Experiment XI_{30}. Outline of tadpole drawn 8 days after the operation. *ab*, line at junction of Rana sylvatica and R. palustris. *oc*, optic cup. × 4 diameters.

FIG. 32. Experiment XI_{74}. Outline 11 days after the operation. The right eye of R. palustris is seen beneath the abdomen of R. sylvatica. × 4 diameters.

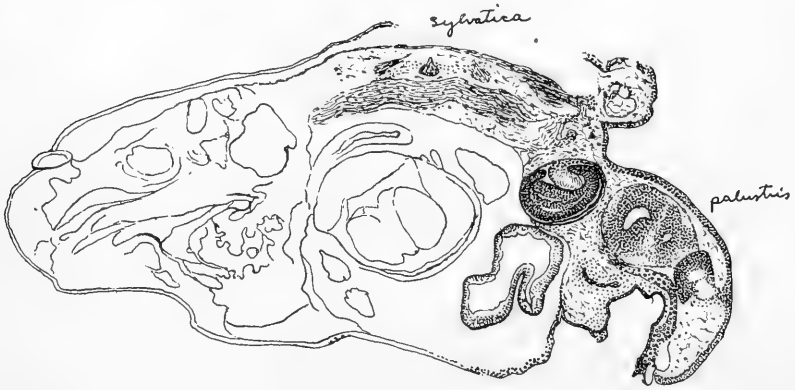

FIG. 33. Experiment XI_{74}. Section through the right eye of R. palustris. × 30 diameters.

Experiment XI_{74}.

As in the other experiments of series XI, the head end of R. sylvatica was grafted on over the right optic vesicle and structures about it of R. palustris. In this experiment, however, the ventral surface of R. sylvatica faces in the same direction as the dorsal surface of R. palustris see Fig. 32. A few days after the operation the right eye of R. palustris became visible beneath the skin of the ventral surface of the abdomen of R. sylvatica near its junction with R. palustris. Eleven days after the operation the tadpole was killed. The sections show a well-developed optic cup with a large, irregular lens, see Fig. 33. The

shape and size of the lens would indicate that it was formed soon after operation and that subsequent growth and shifting of the eye or of the graft or of both brought the eye to lie in the position seen in Fig. 15. The irregular shape of the lens with a long process extending out through the pupil and then bending towards the skin seems to me indicative of such shifting, and a pulling away of the lens from its original position just beneath the skin. This lens is about the same diameter as the normal one on the left side.

Series XII.

In the experiments of series XII a small piece of skin from the abdomen of R. sylvatica was grafted onto the side of the head of R. palustris. As in series XI the skin over the side of the head of R. palustris of stage II was first torn off, leaving the optic vesicle and structures about exposed. Onto this denuded area a thin piece of skin from the abdomen of a slightly older R. sylvatica was grafted. Very thin pieces of skin with scarcely more than the epidermis were found almost impossible to use, as they immediately rolled up into little balls before one could transfer them to the R. palustris. So it was necessary to include a little of the underlying mesenchyme. In most of the experiments the mesenchyme was too thick and prevented the underlying optic vesicle from reaching the ectoderm and in such experiments no lens was formed.

Experiment XII_{51}.

The tadpole of this experiment was killed 7 days after the operation. Fig. 34 shows a lateral view of the tadpole with the irregular mass on the right side of the head. This mass is the grafted-on piece of the abdomen of R. sylvatica. I was not able to determine before sectioning whether the optic vesicle had been in contact with the skin. The sections, however, show an irregular optic cup beneath the skin of R. sylvatica. The skin of R. sylvatica extends on the left from the point *a* to *b*, see Fig. 35. One corner of the optic cup is superficial and between it and the ectoderm is a small lens about 90 micro mm. in diameter, see Fig. 36. The normal one on the left side is about 160 micro mm. in diameter. It is evident that the lens must have come from the ectoderm overlying the optic cup, and consequently from ectoderm from the abdomen of R. sylvatica. We have here then the optic vesicle of R. palustris stimulating the lens formation from the skin from the abdomen of an animal of a different species. This is not more than might be expected when we consider how perfect are the unions between the

grafts of R. palustris and R. sylvatica, and how dependent the lens is for its origin upon the influence of the optic vesicle.

Harrison (see footnote 4) has shown how perfect such unions may be and how the lateral line sense organs starting from the head of R. sylvatica may grow quite normally into the tail of Rana palustris, which has been grafted onto the R. sylvatica.

Eleven other experiments of the series XII in which the embryos were killed in from two to eleven days after the operation show the right eyes deeply buried beneath the surface of the grafted-on ectoderm. In none are there indications of lens formation.

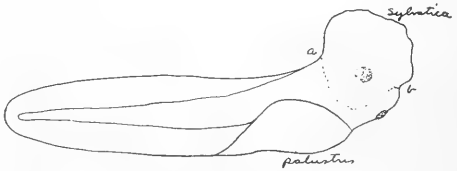

FIG. 34.

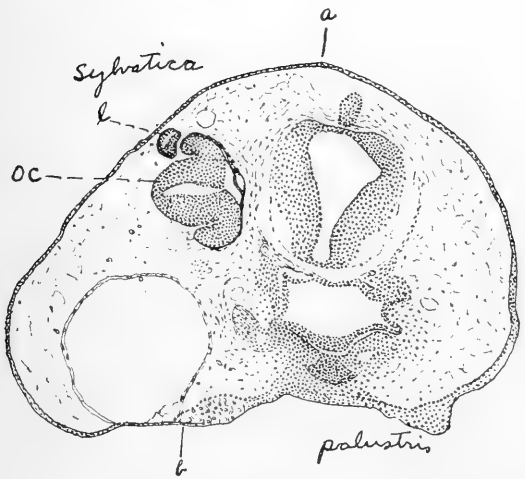

FIG. 35.

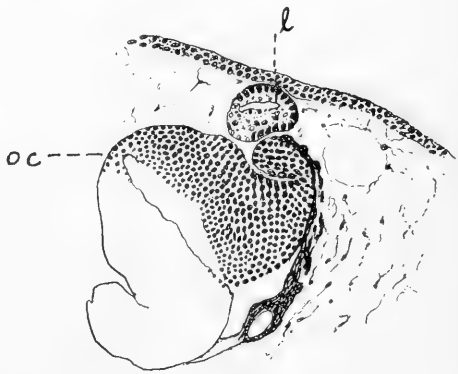

FIG. 36.

FIG. 34. Experiment XII_{51}. Outline 7 days after operation, lateral view. *ab*, line between R. sylvatica and R. palustris, the right eye of R. palustris deeply buried, it approaches nearer the surface of the R. sylvatica ectoderm on the dorsal side. × 4 diameters.

FIG. 35. Experiment XII_{51}. Section through the right eye of R. palustris. *ab*, points at junction of ectoderm of R. palustris and R. sylvatica. *a*, near the middorsal line of R. palustris. *l*, lens. *oc*, optic cup. × 45 diameters.

FIG. 36. Experiment XII_{51}. Enlarged part of Fig. 35. *l*, lens. *oc*, optic cup. × 110 diameters.

The work of Colucci, Wolff, Muller and Fischel on the regeneration of the lens from the edge of the iris might lead one to look for a similar origin in some of the transplanted or deeply buried eyes in the various experiments noted above, but with the exception of the one experiment, DF_{33}, there are no indications of such an origin. The many examples of deeply buried eyes without lenses would lead to the conclusion that

during the early stages of development such an origin would be very uncommon in this species, Rana palustris. I shall show in a subsequent paper that quite the contrary is the case in Rana sylvatica, as in this species the optic cup readily gives rise to a lens from itself if prevented from stimulating one from the ectoderm.

CONCLUSIONS.

These experiments lead to the conclusion that there is no especial predetermined area of the skin which must be stimulated in order that a lens may arise.

They also lead to the conclusion that the lens is dependent for its origin on the contact influence of the optic vesicle on the ectoderm, for we find that when the optic vesicle touches the ectoderm not only under normal conditions but under abnormal ones that a lens arises at the point of contact.

The all-important influence of the optic vesicle is brought out most forcibly by the demonstration of the power it possesses to cause the formation of a lens from ectoderm taken from over the abdomen of a different species.

C. Are Various Portions of the Optic Vesicle Capable of Stimulating Lens Formation?

That various portions of the optic vesicle may stimulate lens formation is clearly indicated by some of the experiments already noted, as where merely a corner of the optic vesicle reaching the skin is sufficient to cause lens formation, see experiments IV_9, XI_{32}, XI_{30} and XII_{51}. This is more clearly shown by those experiments of series DF and IV in which the regenerated optic vesicle formed a small but fairly normally shaped eye with a normally shaped lens. See experiments DF_{55}, Fig. 4; DF_{56}, Fig. 5; IV_5, Fig. 8; DF_{11}, Fig. 16; DF_{45}, Fig. 21; DF_{43}, Fig. 23; DF_{44}, Fig. 25.

Experiment IV_2.

The operation was performed as on the other experiments of this series. Three days after the operation the tadpole was killed. The sections show that the head is considerably flattened on the right side owing to the fact that the right optic vesicle is very small as compared with the one on the left side. One corner of this small regenerated right optic vesicle is in contact with the skin and here a lens is beginning to differentiate from the ectoderm (see Fig. 37). On the left side there is also a thickening of the ectoderm for the lens. The cells of the

right optic vesicle which are in contact with the ectoderm are not the ones which would have normally come in contact with the skin. Compare the sizes of the right and left eyes in Fig. 37, which is drawn in a plane that passes through the center of each eye.

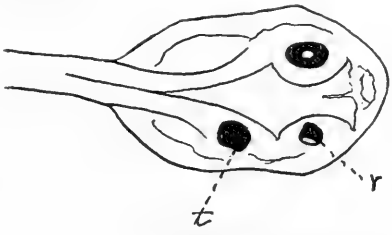

FIG. 38.

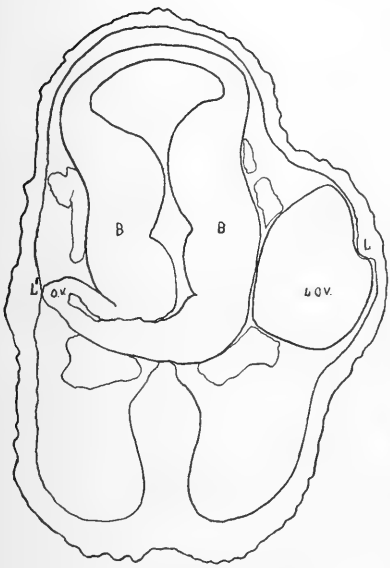

FIG. 37.

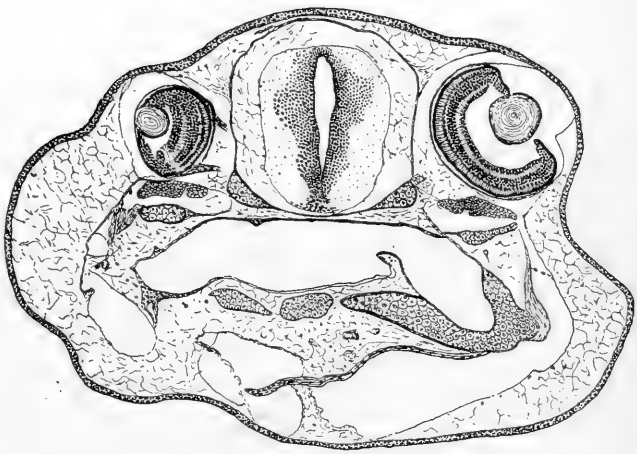

FIG. 39.

FIG. 37. Experiment IV_2. Section through about the center of both eyes. *B*, brain. *L*, left. *L'*, right lens. *OV*, right optic vesicle. × about 70 diameters.

FIG. 38. Experiment IV_{12}. Outline of tadpole killed 11 days after the operation. Drawn in xylol. *t*, transplanted eye. *r*, regenerated eye. × 8 diameters.

FIG. 39. Experiment IV_{12}. Section through about the center of both eyes. × 45 diameters.

Experiment IV_{12}.

The skin flap of an embryo of R. palustris of stage II was turned forward and the optic vesicle cut off close to the brain and consequently that portion of the optic vesicle which would later have become adherent to the skin and stimulated the formation of a lens was entirely removed. Eleven days after the operation the tadpole was killed. Regeneration of the eye had taken place from that portion of the optic vesicle still attached to the brain wall and a small but fairly normally shaped optic cup with a lens resulted, see Figs. 38 and 39. The regenerated optic

vesicle must have reached the skin and stimulated lens formation. The lens over this side is about 110 micro mm. and the normal one about 180 micro mm. in diameter.

Series II.

The experiments of series II differ from those of series DF and IV in that the optic vesicle was destroyed and not transplanted after it was cut off.

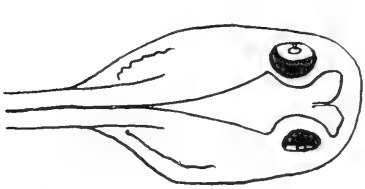

FIG. 40.

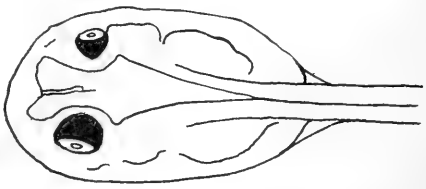

FIG. 41.

FIG. 40. Experiment II_{11}. Outline of tadpole killed 9 days after operation. Drawn in xylol. × 8 diameters.

FIG. 41. Experiment II_{10}. Outline of tadpole. × 8 diameters.

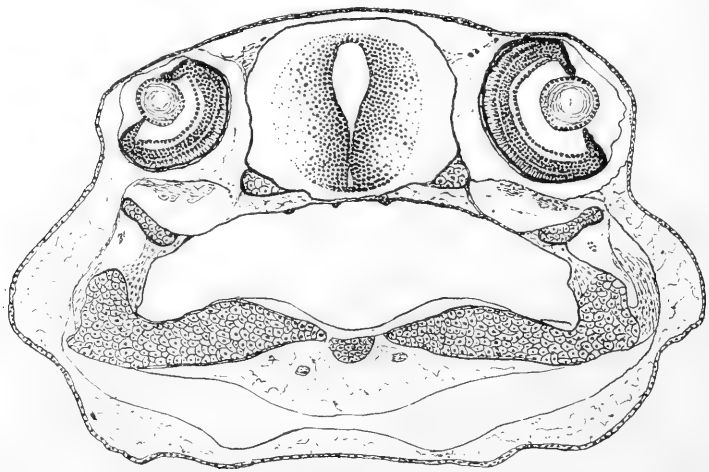

FIG. 42. Experiment II_{10}. Section through about the center of both eyes. × 45 diameters.

Experiment II_{11}.

As in experiment IV_{12}, the optic vesicle was cut off close to the brain of R. palustris, stage II. The skin flap was uninjured and turned back into place, where it readily adhered and healed. Five days after the operation a large reformed optic vesicle with what appeared to be a lens was visible and 9 days after the operation the tadpole was killed. Fig.

40, drawn while the tadpole was in xylol, shows on the right side a regenerated eye with a lens. The eye seems normal in shape and nearly as large as the normal one on the left side. The sections show a normally developed eye except that the optic cup and lens are somewhat smaller in size than the normal one on the left side.

The optic vesicle in this experiment, as in experiment IV_9, was cut off so as to remove all of that portion which would normally have come in contact with the skin and stimulated lens formation. The portion that was left, however, has had the same power of stimulating lens formation.

Experiment II_{10}.

This experiment was performed on the same sized embryo and at the same time as experiment II_{11}. Thirteen days after the operation the tadpole was killed. Fig. 41, an outline of the tadpole when in xylol, shows the small regenerated right eye. Fig. 42, a section through the center of both eyes, shows the small regenerated right eye with a small lens. This eye and lens are apparently both normal except for the smaller size. The right lens is about 130μ and the left lens about 170μ in diameter. In this experiment more of the optic vesicle was probably cut away than in experiment II_{11} or it may have been slightly more evaginated. In either case the amount of material left attached to the brain destined to form the optic vesicle was probably less than in experiment II_{11}. Yet here again a portion of the optic vesicle which was never destined to stimulate lens formation did so.

CONCLUSIONS.

The experiments indicate that various parts of the optic vesicle may stimulate lens formation, which means, I think, that at this early period many or even all of the cells of the optic vesicle or perhaps even all of those which are destined to take part in its formation have the power of stimulating lens formation.

GENERAL CONCLUSIONS.

1. Neither a lens nor a trace of a lens will originate from the ectoderm which normally gives rise to one if the contact of the optic vesicle with the skin is prevented.

2. There is no predetermined area of the ectoderm which must be stimulated in order that a lens may arise. On the contrary various portions of the skin when stimulated by the contact of the optic vesicle may and do give rise to a lens. Not only will a lens arise from various

places on the skin as a result of the contact of the optic vesicle of the same animal, but the optic vesicle of one species may cause a lens to arise from the ectoderm of another species of frog.

3. Various portions of the optic vesicle can stimulate lens formation.

4. We must conclude then that in normal development the lens is dependent for its origin on the contact influence or stimulus of the optic vesicle on the ectoderm.

DISCUSSION.

Are there any conditions under which the lens can arise from the ectoderm without the stimulus of the optic vesicle?

If we include under the term optic vesicle the cells that under normal conditions give rise to a distinct optic vesicle but under abnormal conditions may never evaginate from the brain wall, we can at least say that there is no proof to the contrary. Mencl's [6] case, as Spemann suggests, can best be explained by considering that the optic vesicle cells still form part of the brain wall and that this part of the brain was at one time in contact with the ectoderm, and thus stimulated the lens formation. Rabl's [7] case can easily be explained by a shifting of the ectoderm and newly forming lens away from the small optic vesicle. Schaper's [8] experiments give somewhat similar pictures to Rabl's. The rudimentary lenses in Schaper's experiments were undoubtedly caused by the shifting of the ectoderm and lens, thus removing the newly forming lens from the influence of the optic vesicle. In reality the examples of Mencl and Rabl prove neither one side nor the other and the experimental evidence is all directly for the idea that a lens will not arise from the skin without the stimulus of the optic vesicle cells. Especially does this seem evident if we consider that there is a definite reaction of some kind between the optic vesicle and the ectoderm cells which give rise to the lens.

The fact that the optic vesicle becomes quite firmly adherent to the ectoderm just preceding and during the early stages of lens formation and until after its complete separation from the ectoderm, is very suggestive, indicating perhaps that there is a protoplasmic connection of

[6] Ein Fall von beiderseitigen Augenlinsenausbildung während der Abwesenheit von Augenblasen. Archiv f. Entwickelungsmech., XVI.

[7] Ueber den Bau und die Entwickelung der Linse, I. Zeit. f. Wiss. Zool., Bd. 63, 1898, p. 530.

[8] Ueber einige Fälle atypischer Linsenentwickelung unter abnormen Bedingungen. Anat. Anz., Bd. XXIV, No. 12, Jan., 1904.

some kind between the two tissues, by means of which we can readily imagine that an interchange of protoplasm or of certain substances in the protoplasm may take place. With this interchange there may take place a definite chemical reaction producing within the ectodermal cells new substances which give to them new properties, that of forming a lens being the predominant one. That no new substances flowing from the ectoderm into the optic vesicle cells are necessary for their subsequent differentiation will be shown in another article.

The contact of the optic vesicle and ectodermal cells without interchange of substances may, on the other hand, produce the changes necessary for the lens formation. In whatever manner the change is brought about, it would seem evident that chemical changes have taken place within these lens-forming ectodermal cells, and that the optic vesicle cells by some means have been able to change the chemical nature of these cells, new substances being formed which are now peculiar to the groups of cells that show such a modified development. It would seem probable that the modified development was directly due to these new chemical substances.

It is not unlikely that a new biological chemistry must be developed, its reactions being those between living tissues or between a living tissue and the product of a living tissue. In the example of the lens reaction it seems evident that living ectodermal cells must be acted upon, but it is not so evident that the living optic vesicle cells are necessary, as it may be possible to extract from them substances which will give the lens reaction. By means of such a biological chemistry some of the properties of the various tissues and organs may be discovered and perhaps a deeper insight into the problems of development obtained. The development of a biological chemistry is to be considered as but one of the phases of correlative embryology and in considering the correlations in development the mechanical factors must not be neglected.

There are many more questions in connection with lens formation which suggest themselves. For how long a period does the ectoderm possess the lens-forming property? Are there progressive changes in the ectodermal cells which give to them at the proper time, and only at this period, the power to respond to the stimulus of the optic vesicle? It would seem an easy thing to determine in part at least this question. It seems probable that the epithelial cells of the otic capsule undergo such differentiation as to prevent their response to a stimulus from the optic vesicle leading to lens formation. The several instances in which the transplanted optic vesicle is in direct contact with the otic capsule cells without indication of lens formation would seem to suggest this

and to suggest also that there are at this early stage, shortly after the formation of the otic capsules, chemical differences between the epithelium of the skin and otic capsule. The same question may be applied to the optic vesicle. Before its contact with the ectoderm and again after invagination and differentiation have progressed, does it possess the power to stimulate lens formation? In a subsequent paper I shall show that the optic vesicle when cut away from the brain at an early period and transplanted to other parts of the embryo retains its power of progressive development, of invagination and differentiation of the layers of the retina. Does it lose with these progressive changes its power to stimulate lens formation? Some of the experiments already cited would seem to show that for a short time at least it retains this power.

Does the reaction between the optic vesicle and ectoderm require a definite length of time, and will a decrease in this period influence the development of the lens? After the initial stimulus has been given for lens formation does it become self-differentiating or is continued contact with the optic cup necessary for the normal development of the lens? Normally the lens remains closely adherent to the optic cup until after the former has separated from the ectoderm and only when the vitreous humor begins to form does it separate from the retina. Is this normal attachment necessary for the growth and differentiation of the lens? Will it cease to grow or will it grow abnormally if its normal relations with the optic cup are altered? A few experiments which I have done would seem to indicate that even as late as the time of separation of the lens from the ectoderm the growth of the lens will be retarded if the optic cup is removed. The results of some experiments by Schaper [8] can be explained on the supposition that the reaction between the optic cup and lens is of considerable duration. In Schaper's experiments the lens thickenings were pulled out of the shallow cavity of the optic cup, evidently by the dorsal growth of the ectoderm to cover the large wound resulting from removal of most of the central nervous system. In this dorsal shifting of the ectoderm the lens rudiment was also involved. The lens has thus been removed from its normal intimate relation with the retina with the result that the lens has remained in a very rudimentary condition and in nearly all of his experiments has not even separated from the ectoderm. Schaper's experiments indicate, I think, that the time of the lens reaction is of considerable duration and if disturbed, abnormal development of the lens results.

We have noted in the conclusions that various portions of the ectoderm possess at a certain stage the lens-forming power. Are all por-

tions of the ectoderm at this stage equi-potential or are there only certain areas that have this power? Mencl's theory that the ectoderm of a certain head segment has a tendency to lens formation at a certain developmental stage loses all its meaning when we consider that the lens may arise from abdominal ectoderm. The theory, which has been recently advocated again by Schaper, that the lens is a modified primitive sense organ, will not hold in view of the fact that ectoderm, taken from over the abdomen of R. sylvatica and grafted on over the optic vesicle of R. palustris (see experiment XII_{51}) did not possess at the time of operation the primitive sense organs and yet it gave rise to a lens. Again it seems unlikely that in the several instances in which I have been able to bring about lens formation from strange ectoderm that the optic vesicle should have in each case come in contact with one of these sense organs. And again in such experiments as IV, in which the optic vesicle has never been in contact with the ectoderm which normally gives rise to a lens there is no trace of a rudimentary lens such as Schaper pictures.

Weismann's doctrine of determinents is entirely in opposition to this correlative character of the origin of the lens which is fatal to the view that embryonic differentiation is brought about through qualitative nuclear division during cleavage or at any later stage. It is evident that the lens is not predetermined in the egg and that no such things as "lens-biophores" can exist in the egg nucleus, otherwise we should expect the lens to be self-differentiative from a specific group of ectodermal cells, and from only these cells. The adherents of Weismannism may take the stand that all of the ectodermal cells contain "lens-biophores" which only become active on stimulation by the optic vesicle. Such a position is not to be seriously considered. If it is true that the lens is a correlative product of the ectoderm and optic vesicle it is very probable that many other organs of the embryo likewise arise from the interaction between two or more tissues or organs or their products.

The recent works of Yatsu[9] on Cerebratulus eggs, and of Wilson[10] on Germinal Localization in Dentalium indicates that there are progressive differentiating changes in the unsegmented egg. This progressive differentiation is in the cytoplasm. The mode of formation of the lens gives a clue to these changes. It is easy to imagine that the egg before it leaves the ovary is endowed with a comparatively few specific stuffs

[9] Biol. Bul., 1904.

[10] Jour. of Expt. Zoölogy, Vol. I, No. 1, 1904.

and that through interaction of these new stuffs are formed which become localized in different portions of the egg and during cleavage into certain groups of cells. It is evident that this localization into groups of cells does not prevent farther interaction and formation of more and more specific stuffs, which are the determining components of the various organs and tissues. Thus the specific stuffs which determine the development of the lens are not formed until a comparatively late period in development.

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PROCEEDINGS OF THE ASSOCIATION OF AMERICAN ANATOMISTS.

SEVENTEENTH SESSION.

Wistar Institute of Anatomy, Philadelphia, Pennsylvania, December 29, 30 and 31, 1903.

At its general business session, the association adopted the following recommendations made by the executive committee:

1. That this association express its approval of a project, which consists in the organization of an International Congress of Anatomy to be held in Geneva, in the year 1905, and further that this association express its desire to participate in said Congress.

2. That Charles S. Minot represent this Association as delegate to the International Congress of Anatomy and, in the event that he be unable to attend the meeting of delegates, the president of this association be instructed to appoint a substitute.

The following amendment to Article V of the Constitution, proposed at the session of 1902, was adopted:

"Candidates for membership must be persons engaged in the investigation of anatomical or cognate sciences, and shall be proposed in writing to the executive committee by two members, who shall accompany the recommendation by a list of the candidate's publications together with references."

The following distinguished European anatomists were elected honorary members of this association:

Professor Wilhelm Waldeyer, *Professor of Anatomy, and Director of the Anatomical Institute of the University of Berlin, Germany.*

Professor Carl Toldt, *Professor of Anatomy, University of Vienna, Austria.*

Professor Gustav Retzius, *Professor Emeritus, Stockholm, Sweden.*

Professor Camillo Golgi, *Professor of Histology and General Pathology, Pavia, Italy.*

Twenty-one new members were elected.

The following officers were elected for the ensuing term:

Dr. Charles S. Minot, President.

Dr. George A. Piersol, First Vice-President.

Dr. J. Marshall Flint, Second Vice-President.

Dr. G. Carl Huber, Secretary and Treasurer.

Doctors Franklin P. Mall and George S. Huntington, members of the executive committee.

Dr. Joseph S. Blake, delegate to the Congress of American Physicians and Surgeons.

Professor Simon H. Gage, delegate to the executive committee of the Association for the Advancement of Science.

Treasurer's Report for the Year 1903.

Balance on hand December 26, 1902	$208.73	
Dues and other receipts for 1903	742.90	
	$951.63	$951.63
Expenditures for 1903:		
Expenses of Secretary and smoker at the Washington meeting	$41.81	
The American Journal of Anatomy for 157 subscriptions at $4.50 less 50c	706.00	
Postage and envelopes	24.25	
Printing	52.63	
	$824.69	$824.69
Balance on hand Dec. 24, 1903		$126.94

Audited by a committee consisting of Doctor Daniel S. Lamb and Doctor Frederic H. Gerrish.

ABSTRACTS OF PAPERS PRESENTED.

THE SUPRACOSTAL MUSCLE; ITS OCCURRENCE IN THE MAMMALIAN SERIES, RELATION TO THE HUMAN VARIANTS, AND BEARING ON THE MORPHOLOGY OF THE VENTRO-LATERAL TRUNK MUSCULATURE. Illustrated by diagrams and casts. By George S. Huntington. *Columbia University, New York.*

ON THE HARVARD EMBRYOLOGICAL COLLECTION. By Charles S. Minot. *Harvard Medical School.*

NOTE ON THE CLASSIFICATION OF CERTAIN OF THE FACIAL MUSCLES. By J. Playfair McMurrich. *Anatomical Laboratory, University of Michigan.*

In the B. N. A. list of myological terms there is recognized among the facial muscles a *M. quadratus labii superioris,* credited with three heads of origin, a caput zygomaticum, a caput infraorbitale and a caput angulare. These three heads correspond to what have been elsewhere, and especially in the English text-books, recognized as distinct muscles, the zygomaticus minor, the levator labii superioris proprius and the levator labii superioris alæque nasi.

There can be no question but that the B. N. A. terminology for this group of muscles presents from the æsthetic standpoint decided advantages; but that is not the standpoint from which a classification of muscles should be made. If the morphological standpoint, as is proper, be taken, then it is clear that the B. N. A. grouping is quite artificial since it is known from the observations of Ruge that, while the caput zygomaticum and the caput angulare are derivatives of the superficial layer of the muscle sheet which gives rise to the facial muscles and may be regarded as differentiations of the orbicularis oculi, the caput infraorbitale, on the contrary, is derived from the deep layer and may be regarded as a differentiation from the orbicularis oris.

A BONY SUPRACONDYLOID FORAMEN IN MAN, WITH REMARKS ABOUT SUPRACONDYLOID AND OTHER PROCESSES AT THE LOWER END OF THE HUMERUS. By Thomas Dwight. *Anatomical Department, Harvard Medical School.*

A supracondyloid foramen completely bounded by bone, observed in a white woman, was described. No other such case has been recorded.

The median nerve went through the foramen, the artery over it. This foramen did not have the appearance of an ossification of a band of connective tissue occurring late in life, but was probably laid down in cartilage. The early ossification of the supracondyloid processes points to this as does the shape of the arch in this case and an observation of Tandler's.

A process from the anterior border of the humerus was described, in connection with which was discussed Solger's views, according to which it would be a middle or anterior supracondyloid process. Barkow's external process was discussed, the difficulty of its interpretation considered and the conclusion reached that it is probably of no significance. The difficulty of accounting for supracondyloid processes and similar anomalies on the theory of reversion alone was considered.

THE CAUSE OF INVERSE SYMMETRY. By E. G. Conklin. *Zoological Department, University of Pennsylvania.*

ON THE RELATION OF GASTRIC AND INTESTINAL EPITHELIUM. By Robert R. Bensley. *Hull Laboratory of Anatomy, University of Chicago.*

(Read by title.)

ON THE RELATION OF MOTOR ENDINGS OF NERVES TO THE SARCOLEMMA IN THE MUSCLE OF THE FROG, AND ON THE NATURE OF THE SO-CALLED ULTRA-TERMINAL FIBRILLAE OF RUFFINI. By John G. Wilson. *Hull Laboratory of Anatomy, University of Chicago.*

(Read by title.)

ON THE DISTRIBUTION OF THE ELASTIC TISSUE IN THE HUMAN LARYNX AND ON THE NATURE OF THE ANTERIOR INSERTION OF THE LIGAMENTUM VOCALE. By Dean D. Lewis. *Hull Laboratory of Anatomy, University of Chicago.*

(Read by title.)

THE LOCALIZATION OF THE LECITHIN CONTENT OF THE RED BLOOD CORPUSCLES. By Preston Kyes. *Hull Laboratory of Anatomy, University of Chicago.*

(Read by title.)

THE BRAINS OF THREE BROTHERS. By Edw. Anthony Spitzka. *Columbia University, New York City.*

Opportunities for demonstrating the influence of heredity in the configuration of the human brain are exceedingly rare; adult material of this kind has only once before been described, and by the same writer, before this association three years ago in the case of the brains of the two distinguished physicians, Seguin father and son. It may be remembered that in the Seguin brains there were found some notable resemblances which could be attributed to hereditary transmission. The writer again had the good fortune to test the question of encephalic morphological transmission in the brains of three brothers recently executed together in New York State. In the search for positive evidences of hereditary resemblance, only such parts of the cerebrum as are subject to great range of variation in different brains could be depended upon to support the proposition; it was found, in fact, that peculiarities of anatomical configuration of this class, uncommon enough in the general run of brains as they come to the hands of anatomists, were similarly

reproduced in the three brains. Principal among these are: the unusual form of the paroccipital fissure, confluent with the occipital by the cephalic stipe, separated from the parietal fissure by a slightly submerged paroccipital isthmus, and characterized in each case by an operculation such as the writer had never before seen in quite the same form in any other brain among 200 examined carefully for just this point. Striking similarities also occur elsewhere, as in the separation of the paracentral from the supercallosal, in the form of the postcentral, of the supercentrals and superfrontals, compared according to sides and many other features.

A NOTE ON THE TRUE WEIGHT OF THE HUMAN LUNGS. By Edw. Anthony Spitzka. *Columbia University, New York City.*

In six criminals executed by electricity, the weight of the lungs was found to be much below the averages generally given in the text-books. The differences due to two peculiar conditions attending this mode of death, namely, non-coagulation of the blood and contraction of the vessel walls. With this the sudden closure of the glottis, the contraction of the thoracic cavity, and other phenomena all help to bring about a nearly bloodless condition of the lungs, so that we are enabled to ascertain the actual weight of lung tissue only, not, as in ordinary death, of a variable amount of blood and serum as well.

The table follows:

	Age.	Grams.		Ounces	
		L	R	L	R
Czolgosz	29	220	241	7.75	8.5
Turckofski	41	230	260	8.5	9.5
W. V. W.	27	320	356	11.3	12.5
B. V. W.	23	280	310	9.9	10.9
Gamari	31	216	248	7.6	8.7
Ennis	30	...	269	..	9.5

The weight of the lungs themselves then more nearly approximate 7 and 8 ounces respectively than 20 and 22 ounces as usually given.

THE BIMERIC DISTRIBUTION OF THE SPINAL NERVES IN ELASMOBRANCHII AND URODELA. By Charles R. Bardeen. *The Johns Hopkins University, Baltimore.*

In those vertebrates in which a definite metameric segmentation is maintained in the body-wall, as in the Elasmobranchii and Urodela, the cutaneous nerves of the trunk and tail reach the skin through the myosepta. Each is distributed anteriorly and posteriorly to the myoseptum through which it passes. In Urodela and Elasmobranchii, in those

regions of the body-wall in which the muscle fibers pass from myoseptum to myoseptum, the motor nerves for the muscle fibers are distributed through plexuses lying in the myosepta. Each spinal nerve gives rise to a plexus in the septum through which it sends cutaneous branches to the skin. From the plexus, motor fibers pass to each myotome bordering on the myoseptum. Occasionally a single nerve fiber may be seen dividing and sending one branch to the myotome anterior to the septum and the other to the myotome posterior. As a rule, the muscle fibers are innervated at their extremities where they are attached to the septum. The "basket-like" terminations about the tips of the muscle fibers described by Giacomini (Monit. Zool. Ital. IX, 92-95, 105-110) as sensory endings in the musculature of the body-wall and tail of teleosts, elasmobranchii urodelans and the larvæ of anurans and urodelans were probably motor and not sensory endings. They correspond to the endings recently described by Ceccherelli (Arch. Italiano di Anatomia e Embriologia II, p. 80-86, 1903) on certain muscle fibers of the tongue of the frog. Retzius (Biol. Untersuchungen, III, 1892) has pictured the termination of motor fibers near the extremities of muscle fibers in Elasmobranchii and the bilateral distribution of the fibers of the septal plexus in Myxine glutinosa.

Those morphologists who maintain that each spinal nerve forms with the myotome of its corresponding segment in the vertebrate embryo a neuromuscular union maintained throughout subsequent development should take into consideration the intersegmental position and the bimeric distribution of the motor as well as of the sensory elements of the spinal nerves in vertebrates in which metameric segmentation is strictly preserved in the body-wall.

THE MESONEPHROS OF A THREE WEEKS HUMAN EMBRYO. By Susanna Phelps Gage.

In the specimen under consideration (No. 148 of the Mall collection of Johns Hopkins University), which is of about 19 days' development, the prominent Wolffian ridge shows what is probably the remnant of the pronephros, consisting of a single pronephric funnel with a short duct extending cephalad from it; a series of 16 mesonephric tubules; a condensation of tissue representing the metanephros; and a Wolffian duct which has its beginning at the first mesonephric tubule, separated by a considerable space from the pronephric remnant, and extends to the cloaca.

The mesonephric tubules are in a stage hitherto undescribed in the higher mammals. The first seven present the typical S-shape with

a closed Malpighian corpuscle near the crest of the Wolffian ridge and opening into the duct. The last of this set appears as though just cut off from the coelomic epithelium. The remainder of the tubules do not open into the duct. The first and the fourth to the seventh either open by a clear funnel to the coelom or are continuous with the coelomic epithelium near the crest of the ridge. The second, third and last of the tubules are hollow like the rest but connect neither with the coelomic epithelium nor with the duct. Wax reconstructions were made of the entire Wolffian ridges and of individual tubules. The evidence is positive that there is a transitional condition lying between the two specimens also belonging to the Mall collection described by MacCallum. In one of these (15 days) there is a duct with thorn-like processes and no coelomic funnels. In the other (21 days) all the tubules are of the typical S-shape. A specimen of the Mall collection, of perhaps 17 days, shows a condition approximating that of a kitten and shark which were modeled and in which the tubules, although very simply curved, showed the continuation from coelomic epithelium to the sub-spherical body and from the latter to the Wolffian duct. Such a continuous tubule is not realized in No. 148, a part of the tubules having lost connection with the epithelium of the coelom, a part not having attained connection with the duct.

EPITHELIUM OF THE UTERUS AND FALLOPIAN TUBE IN MAMMALS.

By Simon Henry Gage. *Department of Histology and Embryology, Cornell University.*

A careful examination of text-books in anatomy and histology brings out the fact that the statement is made in all of them that the Fallopian tube or oviduct in mammals is lined throughout by a ciliated epithelium. The statement is equally definite that the epithelium of the entire uterus is also ciliated. Occasionally there are statements concerning differences occurring in certain physiological conditions of the uterus. Whenever anything is said concerning methods, as in Kölliker, 6th ed., vol. III, p. 581, it is stated that the cilia of these ciliated cells are exceedingly difficult of preservation. The present investigation shows: (1) That the oviduct in young mammals is lined by a simple columnar epithelium which may be wholly non-ciliated or there may be a limited number of ciliated cells on the fimbriæ next the ovary. (2) In mature animals, as the bat and the mouse, the beginning of the oviduct, that is the fimbriæ of the pavilion and the folds of the ampulla may be lined by ciliated epithelium, the rest of oviduct may be non-ciliated. In human beings the oviduct, during maturity, appears to be lined through-

out by ciliated cells, but there may be a few non-ciliated cells throughout the whole extent and especially in the isthmus near the uterine ostium.

The uterus was not found lined by a continuous ciliated epithelium in any animal, and not in man. Ciliated cells were found scattered over the surface, sometimes singly and sometimes in small groups. Apparently only about one cell in 15 or 20 was ciliated.

Ciliated cells with their cilia were found no more difficult of preservation in isolation preparations and sections from the oviduct and uterus than from other situations, *e. g.* the trachea.

HEMOLYMPH GLANDS IN DOMESTIC ANIMALS. By Mr. F. G. White. *Department of Histology and Embryology, Cornell University.* (Communicated by Professor Simon H. Gage.)

Besides verifying the presence, as previously reported, of hemolymph glands in man, horse, ox, sheep, pig and rat, I have also found them in cat, rabbit, red squirrel (Sciurus hudsonicus) and chipmunk (Tamais striatus). In the cat they were found in the cephalic portion of the thorax, near the renal vessels, and in the prevertebral region posterior to the kidneys. Five were examined, in each of which these glands were found. In the rabbit very few occur. From the six examined, hemolymph glands were obtained in two cases. In one they were found in the thorax near the carotid arteries, in the other near the vena cava, posterior to the kidneys. In a single red squirrel examined these glands were found near the thyroids, in the thorax near the carotid arteries and in the pelvic region.

In the chipmunk they were found at the branching of the renal vessel. Histological examination was made in every case to determine the true nature of the gland.

In the horse many large hemolymph glands were found in the anterior portion of the thorax, a few between the aorta and dorsal vertebræ, many near the kidneys and a few in the pelvic region and in the mesocolon at its visceral attachment.

Very little difficulty is experienced in identifying, from the gross appearance, hemolymph glands in ox, sheep and pig, as their dark red color forms a marked contrast to the prevertebral fat in which they are found in large numbers. In man and horse one is less sure in thus identifying them, while in the other animals mentioned it is usually necessary to resort to an histological examination.

The glands of the horse offer very favorable material for the study of the histology of these structures. For laboratory purposes, however, material is most easily obtained by getting from the butcher the tra-

chea, heart and lungs of a sheep or calf. The hemolymph glands cannot be missed as they appear like blood clots in the fat. The best staining results were obtained when mercuric chloride was used as the fixing agent. Sections cut in paraffin were stained in saturated alcoholic solution of eosin and then in a saturated aqueous solution of methylene blue made slightly alkaline, dehydrated with neutral 95 per cent and absolute alcohol or with absolute alcohol alone and cleared in xylene and mounted in neutral Canada balsam.

THE DEVELOPMENT AND MORPHOLOGY OF THE URINIFEROUS TUBULES OF CERTAIN MAMMALS. By G. Carl Huber. *Department of Histology and Embryology, University of Michigan.*

THE ODORIFEROUS GLANDS OF THE HUMAN AXILLA. By William Keiller. (Histological report by Dr. M. Charlotte Schaefer.) *Medical Department, University of Texas, Galveston, Texas.*

REPORT OF A CASE OF SUPERNUMERARY MAMMARY GLANDS IN THE AXILLAE OF A WOMAN (with specimens). By William Keiller. *Medical Department, University of Texas, Galveston, Texas.*

THE EMBRYONIC DEVELOPMENT OF THE INTERSTITIAL CELLS OF LEYDIG. By R. H. Whitehead. *University of North Carolina.* American Journal of Anatomy, Vol. III.

A DESCRIPTION OF THE GROSS ANATOMY OF THE ADULT HUMAN BRAIN. By Bern Budd Gallaudet. *Columbia University, New York City.*

Basis of description: Forty adult human brains, upon which gross work only was done. This comprised comparisons with each other of (1) medisections of the diacœle; (2) transections, dorsiventral, along entire dorsal surface of thalamus; and (3) specimens of the thalamus itself, obtained by peeling off "hemisphere" from its lateral surface, and tegmental fibers of mesencephal from its ventral surfaces.

Those points only will be given which are not found in the usual descriptions.

The thalamus is wedge-shaped and has four surfaces, a cephalic border and a rounded caudal extremity, the pulvinar. The surfaces are dorsal, ventral, mesal and lateral.

The cephalic border is the angle of the wedge and is common to both the lateral and mesal surfaces. From it these surfaces diverge caudad. This divergence gives a triangular outline to both the dorsal and ventral surfaces.

The outline of the lateral surface resembles closely that of the mesal surface, as seen in medisections of the diacœle. But its plane is at such an angle (dorsiventral) to that of the mesal surface as to make the area of the dorsal surface almost three times greater than that of the ventral surface. The entire lateral surface is in relation with the internal capsule of the hemisphere.

The mesal surface is the wall of the diacœle. Its ventral border, cephalad, joins the ventral extremity of the cephalic border of the thalamus at an angle situated just laterad to the apex of the tuber or where the infundibulum descends.

The pulvinar is a curved (convexity caudad) four-sided area connecting dorsal and ventral surfaces. The dorsal boundary, the same as the caudal border ("base") of the triangular dorsal surface, is much longer ento-ectally than its ventral boundary, *i. e.* the caudal border ("base") of the triangular ventral surface, because of the angle—already mentioned—of the plane of the lateral surface to that of the mesal surface.

For the same reason, the plane of the lateral boundary of the pulvinar converges dorsiventrad to that of the mesal boundary; while these boundaries themselves are curved (like the pulvinar), each one uniting (caudad) the following borders between surfaces of the thalamus: the lateral one, the border between dorsal and lateral surfaces with that between lateral and ventral surfaces; and the mesal one, the border between dorsal and mesal surfaces with that between mesal and ventral surfaces. Laterad to the lateral boundary of the pulvinar extends a process of thalamus into the ecto (præ) geniculum.

The dorsal surface of the thalamus, caudad, curves into the pulvinar. Cephalad, this surface presents the "anterior tubercle," laterad and mesad to which are the borders between respectively the lateral and dorsal surfaces, and the dorsal and mesal surfaces; while cephaloventrad to which, these borders unite into the cephalic border of the thalamus.

The ventral surface of the thalamus, narrow, triangular and concave ventrad, has for its "base" or caudal border the ventral boundary of the pulvinar. It presents, caudo-cephalad, three areas: (1) The caudal, practically the ento (post) geniculum. (2) The medial, entirely occupied by tegmental fibers. (3) The cephalic, which is that portion of gray matter commonly included in the "tuber cinereum." More exactly, it is the lateral area of the so-called "tuber cinereum," the mesal area of which, the tuber, is part of the floor of the diacœle and is continuous dorsad with the terma. This lateral area of the "tuber cinereum," or cephalic area of the ventral surface of the thalamus, has been observed

by Wilder and called by him simply the "raised unnamed area at either side of the tuber."

The cephalic border of the thalamus as a whole, lies just caudad to the "head" of the cauda striati along the entire curved (concavity caudad) course, ventrad, of the latter to the base of the brain.

TWO SKULLS OF LARVAL NECTURUS. By Robert J. Terry. *Washington University, St. Louis, Mo.*

The skulls described are of larvæ, 49.5 and 22.5 mm. long. The study was begun by Dr. B. F. Kingsbury, who gave his attention for the most part to the development of the bones. When the work was taken up by me a review of the chondrocranium was made in which it was found that the conditions present agreed in most respects with the descriptions of Winslow and Miss Platt for stages of about the same age. It seems to me, however, that there are some indications of the presence of occipital plates, and in regard to the ledge that grows over the facial and palatine nerves and through which the cephalic division of the auditory passes, it appears to me to be the primary floor of the otic capsule in this region, and this view is supported by the fact that the proötic bone in its development extends into this ledge first and not into that cartilage upon which the nerves rest.

The bone called squamosal by Huxley, and likened to a boomerang, appears as a small scale in membrane over the region of the external semicircular canal and overlaps the otic process of the quadrate; a long ligament connects it with the otic operculum. The quadrate ossification is remarkable in having its dorsal half ossify in membrane, while the ventral end is a ring of bone formed in the quadrate cartilage beneath a layer of superficial, loosely-arranged cells.

SOME PRACTICAL ILLUSTRATIONS IN EMBRYOLOGY. By Edmund W. Holmes. *Philadelphia, Pa.*

(Read by title.)

DESCRIPTIONS OF A METHOD FOR PREPARING BRAINS USED IN CLASS DEMONSTRATIONS (with specimens). By Addinell Hewson. *Jefferson Medical College, Philadelphia, Pa.*

These brains were prepared in subjects received at random in different seasons of the year in periods varying from three to ten days after death. Bodies were injected by the following formula, from either the carotids or the femorals:

Arsenate of Soda	2 kilos.
Nitrate of Potash	1 kilo.
Glycerine	2000 cc.
Water	7700 cc.
Formaldehyde (40% solution)	150 cc.
Carbolic Acid (No. 1 Delq. Crystals)	150 cc.

This solution was made by boiling the nitrate of potash, arsenate of soda, carbolic acid and water until the salts were dissolved completely; when cold the glycerine and formaldehyde were added, together with sufficient quantity of thymol to saturate the solution. The body was injected with from two to three gallons of this solution and placed in cold storage and allowed to remain until needed (the arteries having been injected with a starch solution colored with light English vermilion twenty-four to thirty-six hours after the preservative solution was used).

The brains exhibited were removed from the skulls and placed in the above solution minus the glycerine, but with sufficient formaldehyde added to make five per cent. The brains were allowed to remain in this solution from twenty-four to thirty-six hours in order that the formaldehyde might permeate the deeper structures. At the end of this time five per cent more of formaldehyde was added and the brains stored permanently. The effect of such procedure was to present a brain with marked differences in color between the cortex and medullary substance and a sharp definition given to all the ganglia. The brain was in condition to be very readily handled without detriment to its substance, and one of the brains presented had been used in demonstrations and was in good condition still.

The author has used this injection in the preparation of fœtal bodies but could not give any information in regard to the effect upon fœtal brains, but judging from the hardening effect upon the body injected and immersed in the same fluid, the amount of formaldehyde should not be more than one-half per cent.

THE INTRA-EMBRYONIC BLOOD VESSELS OF RABBITS FROM 8½ TO 13 DAYS.

By Frederic T. Lewis. *Harvard Embryological Laboratory.*

From the network of vessels in the splanchnopleure of the yolk sac, all intra-embryonic vessels are apparently derived as offshoots. The network ends medially in the two aortæ. With the formation of the pharynx, this net is so folded as to produce dorsal and ventral aortæ with the connecting first arch. The ventral aorta is continued posteriorly in turn by cardiac vessel and vitelline vein. The vitelline vein then bridges the coelom and sends sprouts forward and backward in the

somatopleure. The forward branch becomes the anterior cardinal vein; the posterior branch, incorporating in its progress prolongations of the intersegmental arteries, becomes the umbilical vein. The umbilical vein sends branches toward the aorta, into the posterior limb and less distinctly into the anterior limb. A longitudinal anastomosis of these vessels, uniting with a sprout from the venous end of the heart, produces the posterior cardinal vein, which then is cut off from the umbilical vein, carrying with it the veins from the limbs. The transverse veins extend from near the duct of Cuvier to the ventral side of the visceral arches, forming a transverse anastomosis. They are found as early in development as the posterior cardinal veins. The superior mesenteric vein develops as a new branch and is not a persistent vitelline vein. The vitelline arteries exist before the segments form and are not segmental structures. The irregular, small arteries around the fourth entodermal pouch do not, as Zimmerman believed, form a distinct aortic arch.

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE EYE IN AMPHIBIA. By Warren H. Lewis. *Department of Anatomy, Johns Hopkins University.*

Spemann's experiments on triton, which were performed by puncturing the eye-spot before closure of the medullary folds, indicate that the lens is dependent on the influence of the optic vesicle on the skin for its origin. For if the eye is destroyed, the lens fails to develop, but if it regenerates and touches the ectoderm a lens is formed, while if it regenerates and remains deeply buried no lens forms. Such deeply buried optic vesicles may invaginate and the optic cup form without the presence of the lens. Without the eye, the clearing of the epithelium for the cornea is absent.

To test these and other points, I employed, during the spring of 1903, a quite different series of experiments. By use of the binocular microscope one can make minute dissections of the living amphibian embryo and can remove various organs, transplant them or alter the normal relations, and so alter the influences they exert on each other. We may thus determine certain correlations necessary to normal development.

The first series of experiments was to turn forward a skin flap from over the optic vesicle of Rana palustris and then to remove the optic vesicle. These operations, as also those in most of the other series, were done before any trace of lens formation was present, that is, shortly after closure of the neural folds. When the optic vesicle failed to regenerate, the lens was absent. If the eye regenerates sufficiently to come in contact with the skin a lens will form. If the regenerated eye is deeply buried, it may invaginate, but a lens does not form.

In a second series of experiments performed as above, the optic cup was transplanted to more caudal portions of the embryo. In one experiment the eye remained superficial and a lens developed between it and the skin. The deeply placed eyes failed to develop lenses, but they continued to grow, invagination and differentiation of the layers of the retina taking place.

In a third series, the skin was completely torn away from over the optic vesicle and a piece of skin from the abdomen of Rana sylvatica grafted onto this denuded area. In one of the experiments, the optic vesicle succeeded in touching the skin and stimulating lens formation. In the other experiments, the optic vesicles remained deep in the mesenchyme but continued to develop, invagination and differentiation of the layers of the retina occurring, but the lens failed to form.

In a fourth series of experiments, the head or tail of half of a slightly older embryo of R. sylvatica was grafted by its cut surface onto the denuded area over the side of the head of R. palustris. The optic vesicle was thus made to project toward the coelom or yolk of R. sylvatica. In most of the experiments, the eye remained deeply buried; in all such a lens failed to develop, but in many, invagination went on and the layers of the retina developed. In a few experiments, the position of the eye altered and it touched the ectoderm, stimulating lens formation from ectoderm near the junction of R. palustris and R. sylvatica.

From these experiments, we may conclude: (1) The lens is absolutely dependent for its origin on the influence of the optic vesicle on the ectoderm. (2) There is no predetermined area of ectoderm which must be stimulated in order that a lens may arise; the ectoderm is probably equi-potential as regards its lens-forming power; more than this even, the ectoderm of R. sylvatica is equi-potential with that of R. palustris in this regard. (3) As after cutting away that part of the optic vesicle which normally stimulates lens formation, the regenerated eyes of various sizes will stimulate lens formation, it seems probable that various portions of the optic vesicle have this power. (4) The lens is not necessary for the invagination of the optic vesicle, nor are attachment to the brain and the normal surroundings at the side of the head necessary.

Although an optic cup forms without the presence of a lens, the chambers of the eye fail to develop and the lack of a vitreous humor is especially noticeable and mesenchyme fills in the small irregular cavity. The absence of a vitreous humor accounts for the small cup cavity, the unexpanded eye and the thick layers of the retina. The same number of layers develop but they are often twice as thick as normal.

The cornea fails to develop when the optic vesicle is entirely removed. Over the regenerated eye with lenses, a cornea develops normally except for size, which is small to correspond to the small regenerated eyes. If the optic cup is torn out after the lens has separated from the skin, a small area of clear epithelium will develop immediately over the undisturbed lens. Such clearing for the cornea will also develop over an optic cup from which the lens has been extracted, but not in all cases.

The vitreous humor develops only when both lens and optic cup are present and in about their normal relations.

We have here one tissue influencing another during the course of development, and from this a new structure, the lens, arises. It seems likely that there is a definite chemical reaction between certain substances of the optic vesicle and certain substances of the ectoderm cells, which results in the formation of new substances within the lens cells, and that these substances give to the lens its peculiar characters and mode of development.

THE HEAD CAVITIES OF CERATODUS FORSTERI. By E. H. Gregory. *Department of Anatomy, University of Pennsylvania.*

(Read by title.)

THE DEVELOPMENT OF THE LUNG OF CHRYSEMYS PICTA. By William S. Miller. *University of Wisconsin.*

If the lung of an adult C. picta be blown up, dried and cut open longitudinally, it will be seen that seven distinct compartments, which for convenience we will call air sacs, can be recognized; four of these air sacs are larger than the other three. The three smaller air sacs are situated on the mesial side of the lung between the first and fourth, which occupy the cephalic and caudal ends of the lung. It will furthermore be seen that as we pass from the anterior to the posterior end of the lung the air sacs become less and less complicated in structure.

The same holds true for the lung in its development; the more anterior the air sac is situated the more complicated is its structure. The fourth, or caudal air sac, although it can be recognized earlier than the fifth, sixth and seventh, is much simpler in structure if studied in its entirety.

In the youngest embryo I have studied, the two primary lung buds had already been formed as hollow outgrowths from the embryonic bronchi; these form the first or most anterior air sac. From the caudal end of this enlargement a bud arises, which gradually grows caudad and forms a nearly straight tube which presents successive enlargements that ultimately develop into the second, third and fourth air sacs.

By the time these four air sacs are established the fifth, or the most anterior of the smaller air sacs, may be seen arising from the mesial side of the bronchus between the first and second air sacs. In rapid succession the sixth and seventh air sacs are formed, the sixth arising from the bronchus between the second and third, while the seventh arises from the bronchus between the third and fourth air sacs.

While this gross division of the lung into air sacs has been taking place, various septa have been formed in the individual air sacs and the characteristic appearance of the adult lung is established.

ON THE ORIGIN AND DESTINATION OF FIBERS OF THE OCCIPITO-TEMPORO-PONTINE BUNDLE (TÜRCK'S BUNDLE, *MEYNERT*). By E. Lindon Mellus. *Department of Anatomy, Johns Hopkins University.*

In a circumscribed experimental lesion of the cortex of the temporal lobe in the monkey, involving the first and second temporal convolutions projection fibers degenerated, passing by way of the sub-lenticular segment of the internal capsule to the pes pedunculi, where they occupy the external fifth (occipito-temporo Brückenbahn, Flechsig: sensory tract, Charcot and others). To reach the pes these fibers break through the inferior portion of the lenticular nucleus in small bundles, pass around the external geniculate body just above the point of exit of the optic tract and enter the pes external to those fibers which form the posterior extremity of the internal capsule as it passes between the thalamus and the lenticular nucleus. Instead of turning downward toward the pons, like the capsular fibers, they pursue a course obliquely backward and slightly downward and, after a very short course in the pes, disappear, apparently passing to the anterior quadrigeminal body.

DEMONSTRATION OF FURTHER INSTANCES OF PARIETAL DIVISION. By Ales Hrdlicka. *United States National Museum.*

THE RELATIVE PROPORTIONS OF THE INTRACRANIAL FOSSAE IN DOLICHOCEPHALY AND BRACHYCEPHALY. By Ales Hrdlicka. *United States National Museum.*

TOPOGRAPHICAL ANATOMY OF THE THORACIC AND ABDOMINAL VISCERA AS TAUGHT IN THE DISSECTING ROOMS OF CORNELL UNIVERSITY AT ITHACA, N. Y. By Abram T. Kerr. *Cornell University.*

(Read by title.)

Topography of the thoracic and abdominal organs is worked out carefully on the dissecting room bodies.

Bodies are embalmed with equal parts 95 per cent carbolic, 95 per cent alcohol and glycerine, which hardens the solid and hollow viscera so that they retain the exact form and relations.

Students make series of drawings showing the relations of the viscera to each other and to the surface. They compare conditions found in the body they dissect with the normal of the text-books and models.

After completing the dissection of the thorax and abdomen, these are studied in series of frozen sections. Each student is supplied with a set of blue prints of the sections upon which he writes the names of the parts as he identifies them.

DEMONSTRATIONS.

1. Charles R. Bardeen, Johns Hopkins University: Specimens illustrating the bimeric distribution of the spinal nerves in elasmobranchii and urodela.
2. Lydia M. DeWitt, University of Michigan. Models of an area of Langerhans.
3. Addinell Hewson, Jefferson Medical College: Specimens of brains for class demonstration.
4. G. Carl Huber, University of Michigan: Models showing the development and form of the uriniferous tubules in certain mammals.
5. William Keiller, University of Texas: *a*. Sections of the odoriferous glands of the human axilla. *b*. Specimens showing the use of carmine gelatine injection in ordinary dissecting room cadavers. *c*. Museum specimens.
6. H. McE. Knower, Johns Hopkins University: *a*. Drawings for anatomical publication. *b*. Specimens illustrating some modifications in the development of the tadpole produced by early removal of the heart rudiment.
7. Warren H. Lewis, Johns Hopkins University: Preparations obtained in experimental studies on the development of the eye of amphibia.
8. Leo Loeb, Department of Pathology, University of Pennsylvania: *a*. Progressive changes of ova in the ovary of the guinea pig. *b*. A hypertrophic variety of atresia of the follicles in the guinea pig.
9. C. F. W. McClure, Princeton University: Exhibit of anatomical preparations from the morphological museum of Princeton University; prepared and mounted by the curator, C. F. Silverton.
10. J. Playfair McMurrich, University of Michigan: A cast of the internal ear prepared after the Von Stein method.
11. E. Lindon Mellus, Johns Hopkins University: Preparations illustrating experimental degeneration of the cortico-temporo-pontine tract.
12. William S. Miller, University of Wisconsin: Models showing the development of the lung in chrysemys picta.
13. Stewart Paton, Johns Hopkins University: The relations of the "Golgi nets" and their relation to the specific grey substance.
14. Florence R. Sabin, Johns Hopkins University: *a*. Models of the human medulla. *b*. Specimens of developing lymph nodes.

15. Edw. Anthony Spitzka, Columbia University: Preparations showing the form of the human heart and the stomach in their contracted conditions.
16. G. L. Streeter, Johns Hopkins University: *a*. Sections showing the myelin sheath of material, first stained in toto, and then embedded in paraffin. *b*. Profile reconstructions showing the development of the nerves in the occipital region in the human embryo, together with control-dissections of the same region in pig embryos.
17. R. J. Terry and J. A. Brown, Washington University, St. Louis, Mo.: Models of the human temporal bone.

CONSTITUTION AND LIST OF OFFICERS AND MEMBERS.

Constitution.

ARTICLE I.

Section 1. The name of the Society shall be the "Association of American Anatomists."

Section 2. The purpose of the Association shall be the advancement of anatomical science.

ARTICLE II.

The officers of the Association shall consist of a President, two Vice-Presidents, and a Secretary, who shall also act as Treasurer. The officers shall be elected by ballot every two years.

ARTICLE III.

The management of the affairs of the Association shall be delegated to an Executive Committee, consisting of seven members, including the President and Secretary, ex-officio. One member of the Executive Committee shall be elected annually.

ARTICLE IV.

The Association shall meet annually, the time and place to be determined by the Executive Committee.

ARTICLE V.

Section 1. Candidates for membership must be persons engaged in the investigation of anatomical or cognate sciences and shall be proposed in writing to the Executive Committee by two members, who shall accompany the recommendation by a list of the candidate's publications, together with the references. The election shall take place in open meeting, a two-thirds vote being necessary.

Section 2. Honorary members may be elected from those not Americans who have distinguished themselves in anatomical research.

ARTICLE VI.

The annual dues shall be five dollars. A member in arrears for dues for two years shall be dropped by the Secretary at the next meeting of the Association, but may be reinstated, at the discretion of the Executive Committee, on payment of arrears.

ARTICLE VII.

Section 1. Five members shall constitute a quorum for the transaction of business.

Section 2. The ruling of the Chairman shall be in accordance to "Roberts' Rules of Order."

Orders Adopted by the Association.

That any change in the constitution of this Association must be presented in writing at one meeting in order to receive consideration and be acted upon at the next meeting; due notice of the proposed change to be sent to each member at least one month in advance of the meeting at which such action is to be taken.

The election of delegates to the Executive Committee of the Congress of American Physicians and Surgeons shall take place every three years.

Newly-elected members must qualify by payment of dues for one year within thirty days after election.

The maximum limit of time for the reading of papers shall be twenty minutes.

The Secretary and Treasurer shall be allowed his traveling expenses and the sum of $10 toward the payment of his hotel bill, at each session of the Association.

That the Association discontinue the separate publication of its proceedings, and that the American Journal of Anatomy be sent to each member of the Association, on payment of the annual dues, this journal to publish the proceedings of the Association, including an abstract of the papers read.

Contributors of papers are requested to furnish the Secretary with abstracts within a fortnight after the meeting.

Past Presidents.

Dr. Joseph Leidy.....1888-1891.
Dr. Harrison Allen.....1891-1893.
Dr. Thomas Dwight.....1893-1895.
Dr. Frank Baker.....1895-1897.
Dr. Burt G. Wilder.....1897-1899.
Dr. George S. Huntington.....1899-1903.

Past Secretaries.

Dr. A. H. P. Leuf.....1888-1890.
Dr. Daniel S. Lamb.....1890-1901.

Officers for 1904.

President.....Charles S. Minot.
First Vice-President.....George A. Piersol.
Second Vice-President.....J. Marshall Flint.
Secretary and Treasurer.....G. Carl Huber.

Executive Committee.

Carl A. Hamann.....Term expiring in 1904.
Lewellys F. Barker.....Term expiring in 1905.
Frederic H. Gerrish.....Term expiring in 1906.
George S. Huntington.....Term expiring in 1907.
Franklin P. Mall.....Term expiring in 1908.

Delegate to Executive Committee of Congress of American Physicians and Surgeons, 1903-6.

Joseph A. Blake.

Alternate.

Frank Baker.

Delegate to the Council of the American Association for the Advancement of Science.

Simon H. Gage.

Member of Smithsonian Committee on the Table at Naples.

George S. Huntington.

For addresses of officers, see list of members.

Honorary Members.

John Cleland.....*Glasgow, Scotland.*
John Daniel Cunningham....*Edinburgh, Scotland.*
Mathias Duval.....*Paris, France.*
Camillo Golgi.....*Pavia, Italy.*
William His.....*Leipzig, Germany.*
Albert von Kölliker.....*Würzburg, Germany.*
Alexander Macalister.....*Cambridge, England.*
L. Ranvier.....*Paris, France.*
Gustav Retzius.....*Stockholm, Sweden.*
Carl Toldt.....*Vienna, Austria.*
Sir William Turner.....*Edinburgh, Scotland.*
Wilhelm Waldeyer.....*Berlin, Germany.*

MEMBERS.

Edward Phelps Allis, Jr., Associate Editor of Journal of Morphology, Milwaukee, Wis. *Menton, France.*

Frank Baker, A. M., M. D., Ph. D., Professor of Anatomy, Medical Department, University of Georgetown, *1728 Columbia Road, Washington, D. C.*

Charles Russell Bardeen, A. B., M. D., Associate Professor of Anatomy, Johns Hopkins University, Baltimore, Md.

Lewellys Franklin Barker, M. B., Professor of Anatomy, University of Chicago, *Quadrangle Club, Chicago, Ill.*

Robert Russell Bensley, A. B., M. B., Assistant Professor of Anatomy, University of Chicago, Chicago, Ill.

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Vilray Papin Blair, A. M., M. D., Lecturer on Descriptive Anatomy and Demonstrator of Anatomy, Medical Department, Washington University, *3525 Lucas Ave., St. Louis, Mo.*

Joseph Augustus Blake, A. B., M. D., Instructor in Surgery, College of Physicians and Surgeons, Columbia University, *601 Madison Ave., New York City.*

John Lewis Bremer, M. D., Harvard Medical School, Boston, Mass.

George Emerson Brewer, A. M., M. D., Instructor in Surgery, College of Physicians and Surgeons, Columbia University, *61 W. 48th St., New York City, N. Y.*

Samuel Max Brickner, A. M., M. D., Gynecologist to Mt. Sinai Hospital Dispensary (N. Y.); Special Student in Anatomy, College of Physicians and Surgeons, Columbia University, *136 W. 85th St., New York City, N. Y.*

J. Fulmer Bright, M. D., Instructor in Anatomy and Assistant Demonstrator of Anatomy, Medical College of Virginia, *408 W. Grace St., Richmond, Va.*

Max Brödel, Artist, The Johns Hopkins Hospital, Baltimore, Md.

William Allen Brooks, M. D., *167 Beacon St., Boston, Mass.*

Frank Irving Brown, A. M., M. D., Instructor in Anatomy, Medical School of Maine, *Box 205, South Portland, Me.*

William Browning, Ph. B., M. D., Professor of Diseases of the Mind and Nervous System, Long Island College Hospital, *54 Leffert Place, Brooklyn, N. Y.*

Joseph D. Bryant, M. D., Professor of Surgery in University and Bellevue Medical College, *32 W. 48th St., New York City, N. Y.*

William Francis Campbell, A. B., M. D., Professor of Anatomy and Histology, Long Island College Hospital, *86 Green Ave., Brooklyn, N. Y.*

Churchill Carmalt, A. B., M. D., Assistant Demonstrator of Anatomy, College of Physicians and Surgeons, Columbia University, *600 Madison Ave., New York City, N. Y.*

William Phillips Carr, M. D., Professor of Physiology, Medical Department, Columbian University, *1418 L St., N. W., Washington, D. C.*

William Guy Christian, M. D., Professor of Anatomy, Medical Department, University of Virginia, Charlottesville, Va.

Benson A. Cohoe, A. B., M. D., Associate in Anatomy, University of Chicago, Hull Laboratory of Anatomy, Chicago, Ill.

Howard Dennis Collins, A. B., M. D., Assistant Demonstrator of Anatomy, College of Physicians and Surgeons, Columbia University, *50 W. 55th St., New York City, N. Y.*

William Merritt Conant, M. D., Instructor in Anatomy in Harvard Medical School, *486 Commonwealth Ave., Boston, Mass.*

Edwin Grant Conklin, A. M., Ph. D., Professor of Zoology, University of Pennsylvania, Philadelphia, Pa.

Eugene Rollin Corson, B. S., M. D., *11 Jones St. East, Savannah, Ga.*

Joseph Davis Craig, A. M., M. D., Professor of Anatomy, Albany Medical College, *12 Ten Broeck St., Albany, N. Y.*

William Darrach, A. M., M. D., Assistant Demonstrator of Anatomy, Cornell Medical School, New York City, *852 Lexington Ave., New York City.*

Alvin Davison, M. A., Ph. D., Professor of Biology, Lafayette College, Easton, Pennsylvania.

Robert H. Mackay Dawburn, M. D., Professor of Surgery and Anatomy, New York Polyclinic Medical School and Hospital, *105 W. 74th St., New York City, N. Y.*

Henry Benjamin Decherd, A. M., M. D., Demonstrator of Anatomy, Medical Department, University of Texas, *1502 Avenue E., Galveston, Texas.*

Francis X. Dercum, M. D., A. M., Ph. D., Professor of Mental and Nervous Diseases, Jefferson Medical College, *1719 Walnut St., Philadelphia, Pa.*

Lydia M. DeWitt, M. D., B. S., Instructor in Histology, Department of Medicine and Surgery, University of Michigan, *925 N. University Ave., Ann Arbor, Mich.*

Franklin Dexter, M. D., *148 Marlborough St., Boston, Mass.*

John Milton Dodson, A. M., M D., Professor of Medicine, Rush Medical College, *568 Washington Boulevard, Chicago, Ill.*

Henry Herbert Donaldson, A. B., Ph. D., Professor of Neurology, University of Chicago, Chicago, Ill.

John Thomas Duncan, M. B., C. M., Professor of Anatomy, Woman's Medical College and Ontario Veterinary College, *45 Bloor St., East, Toronto, Canada.*

Thomas Dwight, M. D., LL. D., Parkman Professor of Anatomy, Harvard Medical School, Boston, Mass.

Robert G. Eccles, M. D., Professor of Organic Chemistry, Brooklyn College of Pharmacy, *191 Dean St., Brooklyn, N. Y.*

Corinne Buford Eckley, Demonstrator of Anatomy in Medical and Dental Departments, College of Physicians and Surgeons, University of Illinois, *979 Jackson Boulevard, Chicago, Ill.*

William Thomas Eckley, M. D., Professor of Anatomy, College of Physicians and Surgeons, University of Illinois; also Dental Department, University of Illinois, *979 Jackson Boulevard, Chicago, Ill.*

Charles Lincoln Edwards, Ph. D., Professor of Natural History, Trinity College, Hartford, Conn.

Gilbert M. Elliott, A. M., M. D., Assistant Demonstrator of Anatomy, Medical School of Maine, Brunswick, Maine.

Charles Andrew Erdmann, M. D., Professor of Anatomy, Medical Department, University of Minnesota, Minneapolis, Minn.

Albert Chauncey Eycleshymer, B. S., Ph. D., Professor of Anatomy, Medical Department, University of St. Louis, St. Louis, Mo.

Harry Burr Ferris, A. B., M. D., Professor of Anatomy, Medical Department, Yale University, *118 York St., New Haven, Conn.*

Joseph Marshall Flint, B. S., A. M., M. D., Professor of Anatomy, Medical Department, University of California, San Francisco, Cal.

John Pierrepont Codrington Foster, A. B., M. D., Lecturer on Anatomy, Yale School of Fine Arts, *109 College St., New Haven, Conn.*

Gilman Dubois Frost, A. M., M. D., Professor of Anatomy, Dartmouth Medical School, Hanover, N. H.

Frank R. Fry, A. M., M. D., Professor of Diseases of the Nervous System, Washington University, *3133 Pine St., St. Louis, Mo.*

Simon Henry Gage, B. S., Professor of Histology and Embryology, Cornell University, Ithaca, N. Y.

Mrs. Susanna Phelps Gage, *Ithaca, N. Y.*

Bern Budd Gallaudet, A. M., M. D., Demonstrator of Anatomy and Instructor of Surgery, College of Physicians and Surgeons, Columbia University, *60 W. 50th St., New York City, N. Y.*

Evelyn Garrigue, M. D., Late Demonstrator of Anatomy, Woman's Medical College, New York, *Colorado Springs, Colo.*

Norman J. Gehring, A. B., M. D., Assistant Demonstrator of Histology, Medical School of Maine, *684 Congress St., Portland, Maine.*

Frederic Henry Gerrish, A. M., M. D., Professor of Anatomy, Bowdoin College, *675 Congress St., Portland, Maine.*

George Lincoln Goodale, A. M., M. D., Professor of Botany, Director of Botanic Gardens, Harvard University, *5 Berkeley St., Cambridge, Mass.*

Milton J. Greenman, Ph. B., M. D., Assistant Director of the Wistar Institute of Anatomy, *36th St. and Woodland Ave., Philadelphia, Pa.*

Elisha Hall Gregory, M. D., Demonstrator of Anatomy, Medical Department, University of Pennsylvania, Philadelphia, Pa.

Carl A. Hamann, M. D., Professor of Anatomy, Medical Department, Western Reserve University, *661 Prospect St., Cleveland, Ohio.*

Irving Hardesty, A. B., Ph. D., Instructor in Anatomy, Medical Department of University of California, San Francisco, Cal.

Jesse Ralph Harris, M. D., *1423 Binney St., Washington, D. C.*

Ross Granville Harrison, Ph. D., M. D., Associate Professor of Anatomy, Johns Hopkins University (*Managing Editor of the Journal of Experimental Zoology*), Baltimore, Md.

Basil Coleman Hyatt Harvey, A. B., M. B., Assistant in Anatomy, Hull Anatomical Laboratory, University of Chicago.

Irving Samuel Haynes, Ph. B., M. D., Professor of Practical Anatomy, Cornell University Medical College, *1125 Madison Ave., New York City, N. Y.*

Charles Morse Hazen, A. M., M. D., Professor of Physiology, Medical College of Virginia, Richmond, *Bon Air, Va.*

John C. Heisler, M. D., Professor of Anatomy, Medico-Chirurgical College, Philadelphia, Pa., *3829 Walnut St., Philadelphia, Pa.*

Charles Judson Herrick, Ph. D., Professor of Zoology, Denison University (*Co-editor of Journal of Comparative Neurology*), Granville, Ohio.

Arthur E. Hertzler, A. M., M. D., Ph. D., Professor of Histology and Pathology, University Medical College, Kansas City, Mo., *Halstead, Kan.*

Addinell Hewson, A. M., M. D., Professor of Anatomy of Philadelphia Polyclinic for Graduates in Medicine; Demonstrator of Anatomy, Jefferson Medical College, *1508 Pine St., Philadelphia, Pa.*

Edmund Wales Holmes, M. D., *2025 Chestnut St., Philadelphia, Pa.*

Grant Sherman Hopkins, D. Sc., D. V. M., Assistant Professor of Veterinary Anatomy, Cornell University, Ithaca, N. Y.

Ales Hrdlicka, M. D., Curator of the Division of Physical Anthropology, Washington, D. C., *United States National Museum, Washington, D. C.*

G. Carl Huber, M. D., Professor of Histology and Embryology, University of Michigan, *333 E. Ann St., Ann Arbor, Mich.*

George S. Huntington, A. M., M. D., Professor of Anatomy, Department of Medicine, Columbia University, *437 W. 59th St., New York City, N Y.*

Clarence M. Jackson, M. S., M. D., Assistant Professor of Anatomy and Histology, University of Missouri, *1201 Paquin St., Columbia, Mo.*

Horace Jayne, M. D., Ph. D., Director of the Wistar Institute of Anatomy, and Professor of Zoology, University of Pennsylvania, *36th St. and Woodland Ave., Philadelphia, Pa.*

William Keiller, L. R. C. P., and F. R. C. S. Ed., Professor of Anatomy, Department of Medicine, University of Texas, Galveston, Texas.

George T. Kemp, M. D., Ph. D., Professor of Physiology, University of Illinois, *Hotel Beardsley, Champaign, Ill.*

Abram T. Kerr, B. S., M. D., Assistant Professor of Anatomy, Department of Medicine, Cornell University, Ithaca, N. Y.

Alfred King, A. B., M. D., Instructor and Demonstrator of Anatomy, Medical School of Maine, *610 Congress St., Portland, Maine.*

J. S. Kingsley, Sc. D., Professor of Biology, Tufts College, Mass.

Henry McE. Knower, A. B., Ph. D., Instructor in Anatomy, Johns Hopkins University (*Co-editor and Secretary of the American Journal of Anatomy*), Baltimore, Md.

Preston Kyes, A. M., M. D., Associate in Anatomy, Hull Laboratory of Anatomy, University of Chicago, Chicago, Ill.

Daniel Smith Lamb, A. M., M. D., Pathologist Army Medical Museum; Professor of Anatomy, Howard University, Medical Department, *Army Medical Museum, Washington, D. C.*

Adrian V. S. Lambert, Assistant Demonstrator of Anatomy, College of Physicians and Surgeons, Columbia University, *126 East 39th St., New York City, N. Y.*

Thomas G. Lee, M. D., Professor of Histology and Embryology, University of Minnesota, Minneapolis, Minn.

Joseph Leidy, Jr., A. M., M. D., Late Assistant Demonstrator of Anatomy and Pathologic Histology, University of Pennsylvania, *1319 Locust St., Philadelphia, Pa.*

Dean D. Lewis, M. D., Associate in Anatomy, Hull Anatomical Laboratory, Chicago University, Chicago, Ill.

Frederic T. Lewis, A. M., M. D., Instructor in Histology and Embryology, Harvard Medical School, *36 Highland Ave., Cambridge, Mass.*

Warren Harmon Lewis, B. S., M. D., Associate in Anatomy, Johns Hopkins University, Baltimore, Md.

William Evan Lewis, M. D., Professor of Anatomy, Miami Medical College, *409 East 5th St., Cincinnati, Ohio.*

John Bruce MacCallum, A. B., M. D., Assistant in Physiology at the University of California, Berkeley, Cal.

William George MacCallum, A. B., M. D., Associate Professor of Pathology, Johns Hopkins University; Resident Pathologist, Johns Hopkins Hospital, Baltimore, Md.

John George McCarthy, M. D., Lecturer and Senior Demonstrator of Anatomy, McGill University, *61 Drummond St., Montreal, Canada.*

George McClellan, M. D., Professor of Anatomy, Academy of Fine Arts, Philadelphia, Pa., *1352 Spruce St., Philadelphia, Pa.*

Charles Freeman Williams McClure, A. M., Professor of Comparative Anatomy, Princeton University, *Princeton, N. J.*

Edward Joseph McDonough, A.B., M. D., Instructor in Histology, Medical School of Maine, *624 Congress St., Portland, Me.*

R. Tait McKenzie, A. B., M. D., Demonstrator of Anatomy, McGill, University, *913 Dorchester St., Montreal, Canada.*

James Playfair McMurrich, A. M., Ph. D., Professor of Anatomy, University of Michigan, *1701 Hill St., Ann Arbor, Mich.*

Franklin P. Mall, M. D., Professor of Anatomy, Johns Hopkins University, Baltimore, Md.

Edward Laurens Mark, Ph. D., LL. D., Hersey Professor of Anatomy and Director of the Zoological Laboratory, Harvard University, *109 Irving St., Cambridge, Mass.*

Walton Martin, Ph. B., M. D., Instructor in Surgery, College of Physicians and Surgeons, Columbia University, *68 East 56th St., New York City, N. Y.*

Rudolph Matas, M. D., Professor of Surgery, Medical Department of Tulane University, *2255 St. Charles Ave., New Orleans, La.*

Washington Matthews, M. D., LL. D., Major and Surgeon U. S. Army (Retired), *1262 New Hampshire Ave., Washington, D. C.*

Edward Lindon Mellus, M. D., *10 East Chase St., Baltimore, Md.*

Clifton Meredith Miller, M. D., Demonstrator of Anatomy and Adjunct Professor of Diseases of the Eye and Ear, Medical College of Virginia, *217 East Grace St., Richmond, Va.*

William Snow Miller, M. D., Assistant Professor of Vertebrate Anatomy, University of Wisconsin, Madison, Wis.

Charles Sedgewick Minot, S. B. (Chem.), LL. D., D. Sc., Professor of Histology and Human Embryology, Harvard Medical School, Boston, Mass.

Samuel Jason Mixter, B. S., M. D., Instructor of Surgery, Harvard Medical School, *180 Marlborough St., Boston, Mass.*

Andrew H. Montgomery, A. B., M. D., Assistant Demonstrator of Anatomy, Cornell Medical College, New York City, *209 W. 107th St., New York City, N. Y.*

Mary Blair Moody, M. D., *Station A, New Haven, Conn.*

Robert Orton Moody, B. S., M. D., Assistant in Anatomy, Hearst Anatomical Laboratory, University of California, San Francisco, Cal.

James Dudley Morgan, A. B., M. D., Clinical Professor, Georgetown University Hospital and Medical School; Physician to Garfield Memorial Hospital, *919 McPherson Square, Washington, D. C.*

John Cummings Munroe, A. B., M. D., Instructor of Surgery, Harvard Medical School, Professor of Surgery, Tufts Dental School, *173 Beacon St., Boston, Mass.*

Burton D. Myers, A. M., M. D., Associate Professor in charge of the Department of Anatomy, Indiana University, Bloomington, Indiana.

Harriet Isabel Noble, M. D., Prosector and Curator of the Department of Anatomy and Instructor in Anatomy, Woman's Medical College of Pennsylvania, *1544 Centennial Ave., Philadelphia, Pa.*

Henry Fairchild Osborn, Sc. D., LL. D., DaCosta Professor of Zoology in Columbia University; Curator of Vertebrate Paleontology, American Museum of Natural History, *77th St. and Eighth Ave., New York City, N. Y.*

Charles Aubrey Parker, M. D., Instructor in Anatomy and Instructor in Surgery, Rush Medical College, University of Chicago, *18 S. Homan Ave., Chicago, Ill.*

Stewart Paton, A. B., M. D., Associate in Psychiatry, Johns Hopkins University; Director of Laboratory, Sheppard and Enoch Pratt Hospital, *213 West Monument St., Baltimore, Md.*

William Patten, Ph. D., Professor of Zoology and Head of the Department of Biology, Dartmouth College, *Hanover, N. H.*

Richard M. Pearce, Jr., M. D., Director of Bender Laboratory, Adjunct Professor of Pathology and Bacteriology, Albany Medical School, Albany, N. Y.

George A. Piersol, M. D., Professor of Anatomy, University of Pennsylvania, *4724 Chester Ave., Philadelphia, Pa.*

Eugene H. Pool, A. B., M. D., Assistant Demonstrator of Anatomy, College of Physicians and Surgeons, Columbia University, *57 W. 45th St., New York City, N. Y.*

Alexander Primrose, M. B., C. M. Ed., M. R. C. S. Eng., Professor of Anatomy, University of Toronto, *100 College St., Toronto, Canada.*

Albert Moore Reese, A. B., Ph. D., Associate Professor of Histology and Embryology, Syracuse University, Syracuse, N. Y.

Emory William Reisinger, M. D., Assistant in Anatomy at the Georgetown Medical and Dental Schools, *1209 13th St., N. W., Washington, D. C.*

Daniel Graisberry Revell, A. B., M. B., Associate in Anatomy, University of Chicago, *667 E. 62d St., Chicago, Ill.*

Nelson G. Russell, M. D., Assistant in Anatomy, Medical Department, University of Buffalo, *448 Franklin St., Buffalo, N. Y.*

Florence R. Sabin, B. S., M. D., Associate in Anatomy, Johns Hopkins Medical School, Baltimore, Md.

Marie Charlotte Schaefer, M. D., Lecturer and Demonstrator of Biology, Normal Histology and Biology, Medical Department, University of Texas, Galveston, Texas.

Harold D. Senior, M. B., F. R. C. S., Demonstrator of Anatomy, Medico-Chirurgical College, *10 South 18th St., Philadelphia, Pa.*

George E. Shambaugh, Ph. B., M. D., Instructor in the Anatomy of the Ear, Nose and Throat, University of Chicago; Associate in Osteology, Rush Medical College, *100 State St., Chicago, Ill.*

Francis John Shepherd, M. D., C. M., M. R. C. S. Eng., Professor of Anatomy in McGill University, *152 Mansfield St., Montreal, Canada.*

Daniel Kerfoot Shute, A. B., M. D., Professor of Anatomy, Columbia University, *1101 13th St., N. W., Washington, D. C.*

Richard Dresser Small, A. B., M. D., Instructor in Anatomy, Portland Medical School, *154 High St., Portland, Me.*

Charles Dennison Smith, A. M., M. D., Professor of Physiology, Bowdoin College; Instructor in Physiology and Clinical Microscopy, Portland Medical School, *126 Free St., Portland, Me.*

Eugene Alfred Smith, M. D., *1018 Main St., Buffalo, N. Y.*

John Holmes Smith, M. D., Professor of Anatomy, University of Maryland, *27 W. Preston St., Baltimore, Md.*

Edmond Souchon, M. D., Professor of Anatomy and Clinical Surgery, Tulane University, *2403 St. Charles Ave., New Orleans, La.*

Edward Anthony Spitzka, M. D., Fellow in Anatomy, Columbia University, *66 East 73d St., New York City, N. Y.*

John Anderson Springle, M. D., C. M., Lecturer on Anatomy, McGill University, *1237 Dorchester St., Montreal, Canada.*

George David Stewart, M. D., Professor of Anatomy and Clinical Surgery, University and Bellevue Hospital Medical School, *143 East 37th St., New York City, N. Y.*

George L. Streeter, M. D., Assistant in Anatomy, Johns Hopkins University, Baltimore, Md.

Mervin T. Sudler, M. D., Ph. D., Instructor in Anatomy, Cornell University, *Gen'l Memorial Hospital, 106th St. and Central Park, West, New York City.*

Ewing Taylor, A. B., M. D., Austin Teaching Fellow in Histology and Embryology, Harvard Medical School, Boston, Mass.

Robert James Terry, A. B., M. D., Professor of Anatomy, Medical Department, Washington University, *1806 Locust St., St. Louis, Mo.*

William C. Thro, A. M., Instructor in Histology and Embryology, Cornell University, *Ithaca, N. Y.*

Walter E. Tobie, M. D., Assistant Demonstrator of Anatomy, Medical School of Maine, *126 Free St., Portland, Me.*

Paul Yoer Tupper, M. D., Professor of Applied Anatomy and Operative Surgery, Medical Department, Washington University, *Linmar Building, St. Louis, Mo.*

Albert Henry Tuttle, M. Sc., Professor of Biology, University of Virginia, *Charlottesville, Va.*

Arthur S. Vosburgh, A. B., M. D., Assistant Demonstrator of Anatomy, Medical Department, Columbia University, *40 West 88th St., New York City, N. Y.*

Frederick Clayton Waite, A. M., Ph. D., Assistant Professor of Histology and Embryology, Western Reserve University, *St. Clair and Erie Sts., Cleveland, Ohio.*

Charles Howell Ward, Director of Anatomical Laboratory for the Preparation of Osteological Specimens and Anatomical Models, *47 Hope Ave., Rochester, N. Y.*

John Warren, M. D., Demonstrator of Anatomy, Harvard Medical School, Boston, Mass.

John Clarence Webster, B. A., M. D., F. R. C. P. Ed., Professor of Obstetrics and Gynecology, Rush Medical College, University of Chicago, *706 Reliance Bldg., 100 State St., Chicago, Ill.*

Faneuil D. Weisse, M. D., Professor of Anatomy, New York College of Dentistry, *46 West 20th St., New York City, N. Y.*

Charles Ignatius West, M. D., Lecturer on Topographical Anatomy, Howard University, and First Assistant Surgeon, Freedman's Hospital, *924 M St., N. W., Washington, D. C.*

Richard Henry Whitehead, A. B., M. D., Professor of Anatomy, Medical Department, University of North Carolina, Chapel Hill, N. C.

Burt G. Wilder, M. D., B. S., Professor of Neurology, Vertebrate Zoology and Physiology, Cornell University, Ithaca, N. Y.

Samuel Wendell Williston, M. D., Ph. D., Professor of Paleontology, University of Chicago, Chicago, Ill.

J. Gordon Wilson, M. D., Associate in Anatomy, University of Chicago, *5842 Rosaline Court, Chicago, Ill.*

William Powell Wilson, B. S., Sc. D., Director of the Philadelphia Commercial Museum, *233 South 4th St., Philadelphia, Pa.*

Frederick Adams Woods, M. D., Instructor in Histology and Embryology, Harvard Medical School, *936 Beacon St., Brookline, Mass.*

George Woolsey, A. B., M. D., Professor of Anatomy and Clinical Surgery, Cornell University Medical College, *117 East 36th St., New York City, N. Y.*

Simon M. Yutzy, M. D., Senior Demonstrator of Anatomy, University of Michigan, *Ann Arbor, Mich.*

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