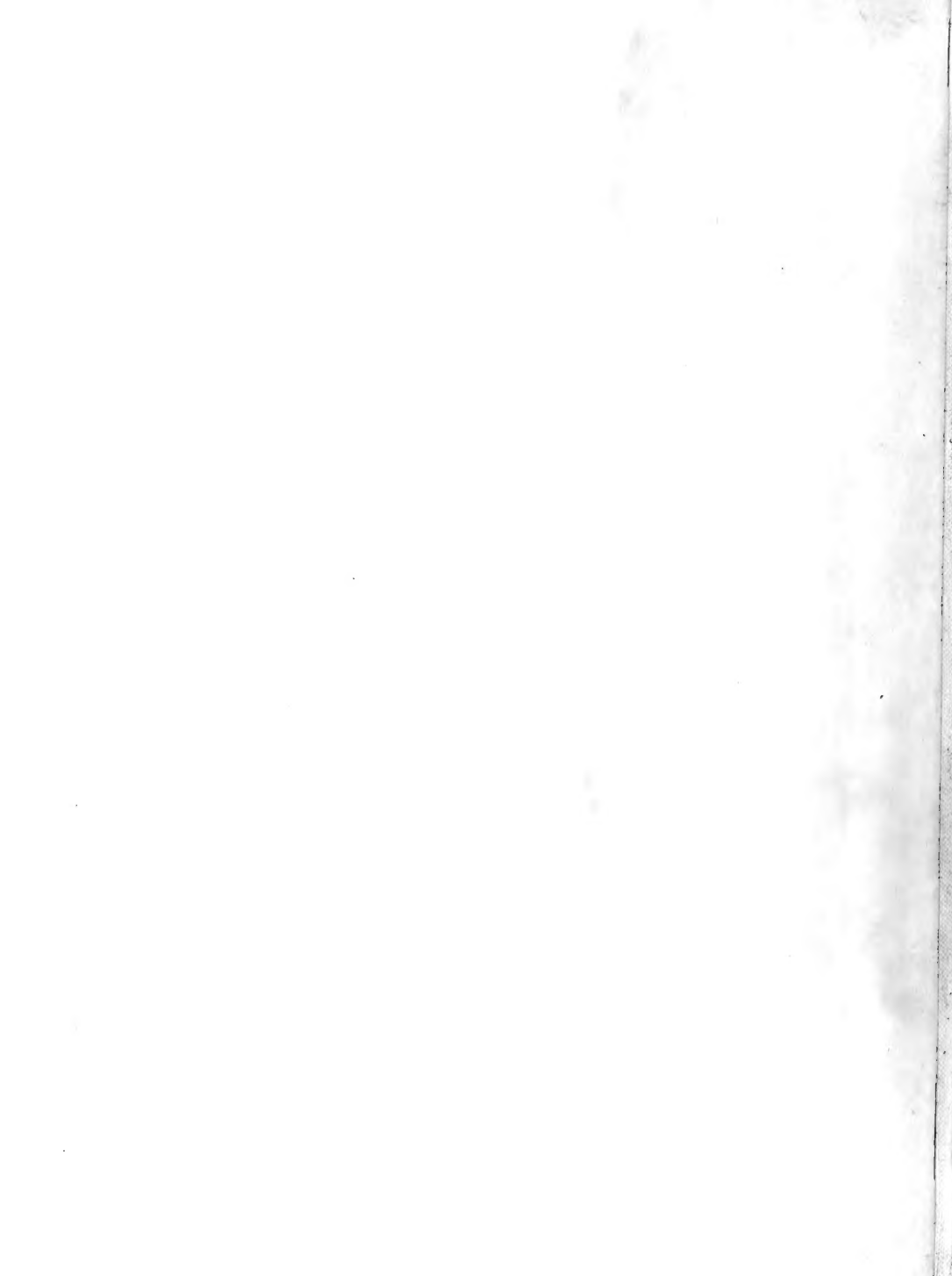
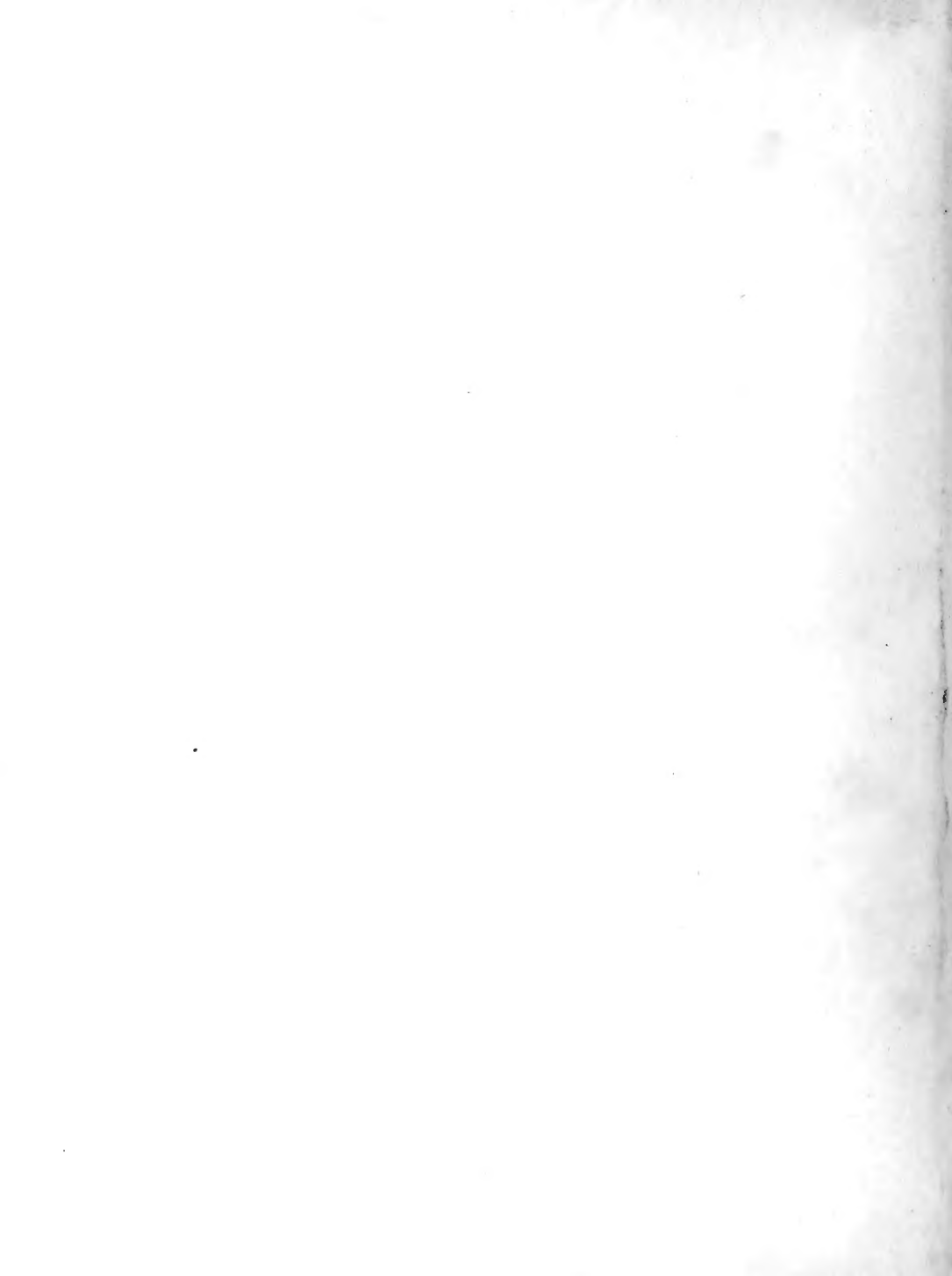


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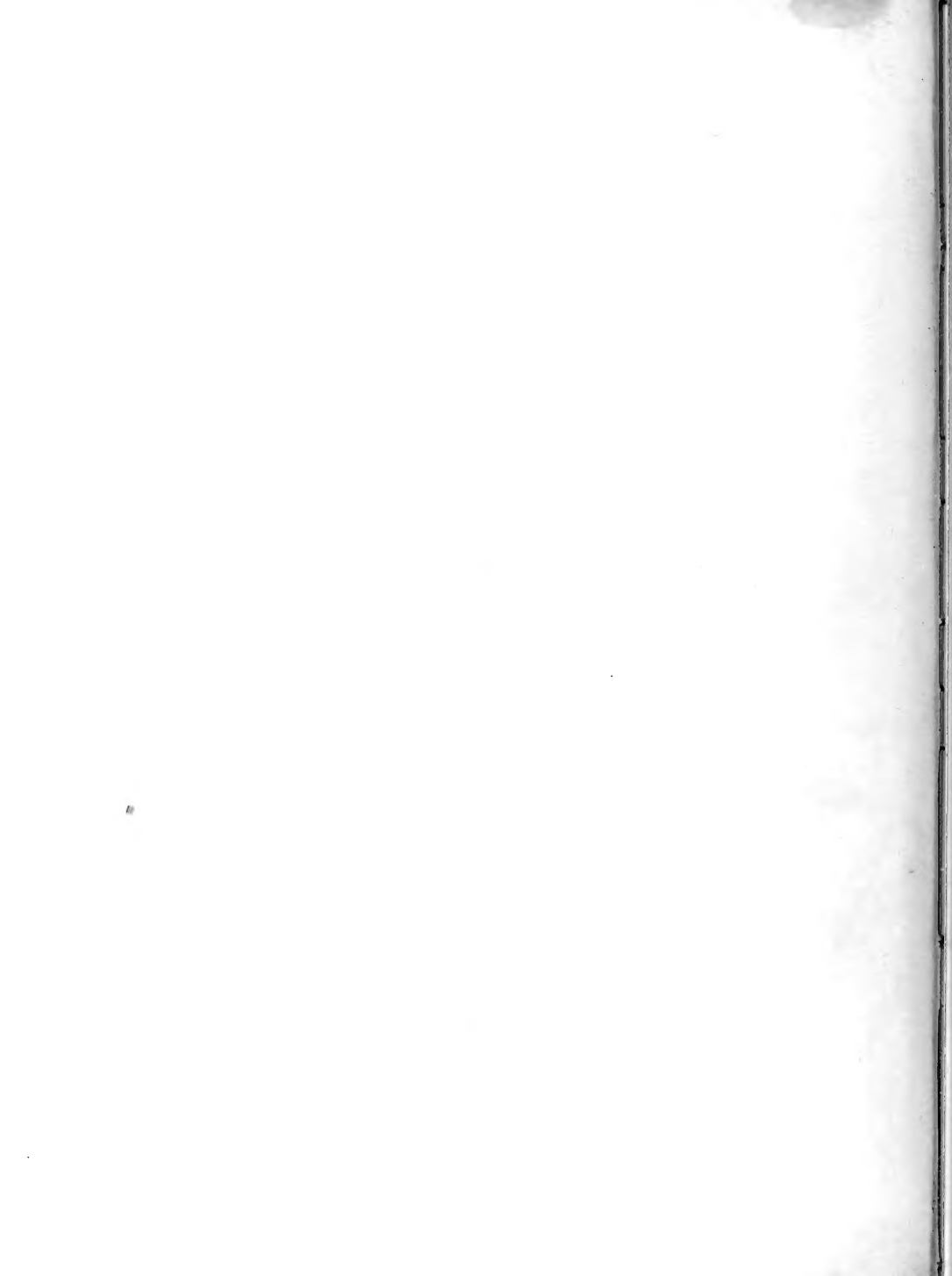
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CONTRIBUTIONS TO EMBRYOLOGY, No. 20.

**THE HISTOGENESIS AND GROWTH OF THE OTIC CAPSULE AND ITS
CONTAINED PERIOTIC TISSUE-SPACES IN THE
HUMAN EMBRYO.**

BY GEORGE L. STREETER.

With four text-figures and four plates.

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THE HISTOGENESIS AND GROWTH OF THE OTIC CAPSULE AND ITS CONTAINED PERIOTIC TISSUE-SPACES IN THE HUMAN EMBRYO.

BY GEORGE L. STREETER

INTRODUCTION.

During the past year the writer has published two shorter communications regarding the development of the connective tissue and cartilaginous coverings that inclose the membranous labyrinth, one of which dealt with the histogenesis of the cartilaginous capsule and the other with the periotic tissue-spaces that are formed in the interval between the cartilaginous capsule and the membranous labyrinth. In the present paper the same matter will be treated in more complete form and a general description will be given of the development of the otic capsule as a whole and of the problems involved in its growth.

In making this study the effort has not been so much toward the determination of the exact form of the capsule as it has been toward the detection of some of the factors that are involved in the production of this form. These two problems, however, are not to be altogether separated. It is the distinctive form of the otic capsule that makes it a particularly favorable place for determining the histological features of the growth of such a structure. Owing to the fact that it is so well provided with known landmarks, the changes in its size and form can be accurately followed and it is therefore possible to determine deductively at what points, for instance, new cartilage is being laid down and at what points it is being removed.

It was soon recognized that the growth of the otic capsule resolves itself into an increase in its external dimensions and a simultaneous hollowing-out and reshaping of its contained cavities, the latter being so managed that their general form and proportions are continuously maintained and a suitable space always provided for the enlarging membranous labyrinth. It is particularly the feature of cartilage excavation accompanying the increase in the total mass to which attention will be invited. It is quite evident that such growth can not be explained on the basis of a simple interstitial increase in the amount of cartilage, together with its passive rearrangement to allow for the enlarging cavities, due, one might say, to a mechanical expansive pressure from the growing membranous labyrinth with its surrounding tissue and fluid. Such a passive rearrangement could only occur in a tissue that is very plastic, whereas cartilage is one of the least plastic of the embryonic tissues. Moreover, the histological picture is not that of mechanical pressure; the cartilaginous chambers are always excavated slightly in advance of the space actually required by the membranous labyrinth, and there is no evidence of the labyrinth being cramped or of the creation of pressure grooves in the margin of the cartilage.

Furthermore, it can not be the perichondrium that is the essential factor, either in the deposit of new cartilage or in the excavation of the old, because the perichondrium, as we shall see, is not formed until after a considerable amount of the growth and hollowing-out of the labyrinth is already completed. Therefore, in the development of the cartilaginous capsule there is something more than interstitial and perichondrial growth.

As forming at least one element, and an important one, in this process it has been found that there occurs a regression of certain areas of cartilaginous tissue to a more embryonic form followed by its alteration into a different type of tissue. It is this process of dedifferentiation that constitutes the essential factor in the hollowing-out and reshaping of the otic capsule which take place continuously during its development. Though the significance and wide occurrence of dedifferentiation and redifferentiation have been well known to botanists and to those investigators who have worked with the simpler forms of animal life, this, as far as the writer knows, is the first time that they have been shown to occur in the human embryo. It is not unlikely that these principles will eventually enter into our conception of the growth of other tissues and organs in human as well as in other mammalian embryos. The establishment of this point, of the occurrence of retrogressive as well as progressive differentiation in human embryos, is considered by the writer to be the chief contribution of the following paper.

The fate of the periotic connective tissue that intervenes between the cartilage and the membranous labyrinth and the formation of the characteristic periotic spaces form problems that are naturally of a morphological character. These spaces have been studied by modeling methods and a description will be given of the steps by which the larger spaces acquire their adult form. It will be pointed out that these spaces show a marked individuality. They have constant and definite characteristics, including their time and point of origin, the manner in which they spread, and their eventual form and structure. They have a structural individuality which, though less complicated, is just as definite as that of the other parts of this sense-mechanism. All of this we will come to later.

TERMINOLOGY.

The writer is not unmindful of a certain feeling of distress that is aroused when it is found on reading a new paper that the author of it is adding to the already difficult matter of following another's description by making a new application of terms or by introducing a whole battery of freshly created ones. Nomenclature constitutes one field in which rock-bound conservatism has many points of merit and where originality may expect a cold and critical reception. It is therefore with some embarrassment that the writer approaches the subject of terminology, and it is also with some apprehension as to whether the "originality" in this instance will prove to be justified. It has in fact seemed best to avoid the incorporation of the term "lymphatic" in describing of the tissue-spaces surrounding the membranous labyrinth. It has been the custom to designate these as "perilymphatic" spaces since 1833, when the term was introduced by Breschet, who thus distinguished

them from the "endolymphatic" cavities of the membranous labyrinth. These terms, together with the terms "perilymph" and "endolymph" for their contained fluids, seemed particularly appropriate and in practical use have proved to be very convenient. Since Breschet's time, however, the lymphatic vascular system has taken on an increased and individual importance, due to researches in which American investigators have taken a particularly active part, and it now seems important to restrict the term "lymphatic" to it and its associated structures.

Inasmuch as the tissue-spaces surrounding the labyrinth have no known connection with the true lymphatic system, either in their origin or in their ultimate relations, it follows that the use of the term "lymphatic" in connection with them is misleading. It therefore seems advisable to eliminate it, even at the expense of losing such a convenient terminology. As a substitute for "perilymphatic" the term "periotic" was finally decided upon and will be so used throughout this paper. In the formation of this adjective the Greek word *ὄτος*, from which it is derived, is used in the restricted sense of representing the essential sense-organ, that is, the otocyst itself and eventually the membranous labyrinth. Inasmuch as numerous words derived from the same source are in common use, it is felt that this term will be readily understood.

We shall speak of a periotic connective tissue that everywhere surrounds the membranous labyrinth. This periotic connective tissue includes in part the fine-meshed periotic reticulum, and in part the large walled-off periotic spaces with their contained periotic fluid, the most prominent of which are the scala vestibuli, scala tympani, and the vestibular cistern. For the term "endolymphatic" fluid one could substitute "otic" fluid; we would then have "liquor perioticus" and "liquor oticus." In all other instances, except when elsewhere specified, the Basillensis Nomina Anatomica terms have been adhered to. The term "semicircular duct" is used to specify the epithelial or membranous canal as distinguished from the cartilaginous semicircular canal. This usage was recommended by Breschet and was adopted in the BNA. It was not taken advantage of, however, by Retzius (1884) in his monograph on the vertebrate ear, who used the term "semicircular canal" for the epithelial channel as well as for the cavity in which it lies. The influence of this great monograph has delayed somewhat the adoption of the BNA recommendation, and one finds subsequent writers still following Retzius in this respect, among whom may be mentioned v. Ebner, R. Krause, Röthig, and myself in previous papers on the development of the membranous labyrinth. In a similar manner the usage by Retzius of the term "anterior" canal instead of "superior" canal, as recommended by the BNA, has occurred in relatively recent papers, including, it must be confessed, those of my own. In the present paper, however, this usage has been corrected. In the historical review which follows the various structures mentioned will be largely referred to in the older terms used by the respective authors.

HISTORICAL.

The first monographic treatise on the anatomy and physiology of the ear was that published by Breschet in 1833. This work proved to be a very important one, both as regards the new observations contained in it and the constructive manner in which the facts then known were analyzed. The terminology of the ear region was standardized and most of the names that were used or introduced by Breschet are in use in the literature of to-day. Before stating his views concerning the structures with which we are dealing, reference must be made to the work of some of his predecessors, and this will be given essentially as outlined by him.

The early anatomists were familiar with the bony labyrinth, but supposed that the spaces contained within it were filled with air. In 1707, however, Valsalva described the normal presence of a fluid in the labyrinth which he compared to the fluid seen in serous cavities. The presence of this fluid was confirmed by Vieussens (1714). His observations were made chiefly on new-born infants, in which he studied the distribution of the labyrinthine fluid and found it present in the vestibule, the cochlea, and in the semicircular canals. The same fluid was also referred to by Cassebohm (1735) and Morgagni (1740). Up to that time no author had directed any particular attention to the labyrinthine fluid, nor had anyone attempted to assign any function to it other than that of moistening the auditory nerve. It was Cotugno (1768) who first endeavored to show that the labyrinthine fluid had some connection with the transmission of sound vibrations. He maintained that there was no air in the spaces of the labyrinth, but that it was everywhere filled with the fluid, which according to his description exudes from the ends of the capillary arteries that are distributed throughout the membrane that lines the cavity of the labyrinth. He described the fluid as being drained off by means of the two aqueducts. Because of the completeness of his description and the interest which he attracted toward the subject, the labyrinthine fluid was thereafter known for more than half of a century as Cotugno's fluid.

Any further advance regarding the nature of the labyrinthine fluid required a more detailed knowledge of the soft parts of the labyrinth. Nerve-like cords and semicircular tubes had been seen in the canals and membranous partitions and sacs had been seen in the vestibule, but it remained for Scarpa (1789) to establish the identity of the membranous labyrinth. He showed that in man and other mammals the semicircular tubes and the vestibular sacs are of the same nature and form one system, and that they are distinct from periosteum. He described how they open freely into each other and are filled with a limpid fluid which distends them. This fluid was thereafter referred to as the fluid of Scarpa. He recognized it in a general way as distinct from the labyrinthine fluid, in which all parts of the membranous labyrinth floated, but otherwise grants it no further attention.

The relations and significance of the fluid of Cotugno and the fluid of Scarpa were not completely recognized until the publication of the noteworthy monograph of Breschet (1833), of which we have spoken. He introduced the terms perilymph and endolymph, by which they have since been known. The existence of the cochlear duct was unknown to Breschet, but otherwise his description of the labyrinth spaces and their contained fluids is the foundation on which the more recent descrip-

tions are principally based. He showed that the perilymph occupies all the space of the bony labyrinth that is not taken up by the semicircular tubes, the utricle (median sinus), and the saccule. It surrounds these everywhere and separates them from the bony walls of the labyrinth. The perilymph also, according to him, fills the spaces of the cochlea and circulates freely throughout the whole system. The scala tympani is connected at its apical extremity with the scala vestibuli by means of the opening to which he gave the name helicotrema. The scala vestibuli in turn opens freely into the vestibule, into which also the semicircular tubes open. He points out the fact, and discusses its relation to the mechanism of hearing, that any vibrations transmitted to the perilymph by the foot-plate of the stapes would be transmitted freely and evenly to the whole of the membranous labyrinth and to the lamina spiralis. He describes the perilymph as consisting of a thin, watery, saline fluid containing a small amount of albumin. He believed that it was secreted by the thin, delicate membrane lining the cavity of the labyrinth and that the materials were brought there by the small blood-vessels that supply this layer. The aqueducts, according to him, are not for the transmission of perilymph, but only serve as passages for veins. He regards them of embryological significance; that they represent the remaining strands of connection with the dura mater, of which the inner ear is a part that has been separated off by the enveloping growth of bone. A description is given of the distribution of perilymph in different animals and it is pointed out that in some fish it communicates with the fluid surrounding the central nervous system and how in such cases the oily or gelatinous cerebro-spinal fluid actually serves as the perilymph. Entirely distinct from the perilymph is the endolymph, which is the fluid filling all parts of the membranous labyrinth.

Breschet describes the character of the endolymph in different animals. He shows that it always contains calcareous deposits, which he designates as otoliths and otoconia, depending on whether they are in the form of lumps or dust. He shows that these are distributed at definite places, at the points of nerve-terminations, and suggests that they act as dampers that tend to check the prolonged vibration of the endolymph. In comparing the ear with the eye, he suggests that the perilymph bears the same relation to the organ of hearing that the aqueous humor bears to the organ of vision. The vitreous body he considers analogous to the endolymph.

Special emphasis has been given to the treatise of Breschet because it marks the beginning of the modern epoch in the anatomy of the ear. Previously the descriptions of this organ had been purely fragmentary. Breschet's monograph is both comprehensive and analytic. If his treatise is searched for defects that are revealed in the light of our present knowledge of the anatomy of the ear, one would name perhaps only two major ones. One of these concerns the inaccurate and meager nature of the embryological features as given by him, and the other concerns the membranous cochlea, the existence of which was entirely unknown at that time. It is interesting to note that these two defects prove to be related; that it was through embryological investigations that the membranous cochlea was eventually discovered.

At about the time of Breschet's treatise, Huschke (1831) made the discovery that the membranous labyrinth was originally a pit in the skin, a fundamental point

that was confirmed later by other embryologists. He also found that in sheep and calf embryos the lamina spiralis is hollow, constituting a spiral tube that is closely attached to the bony walls of the cochlea. He evidently had before him the ductus cochlearis. He supposed, however, that the scalæ in their formation flattened out this hollow tube, thereby converting it into the lamina spiralis, and thus he did not grasp the meaning of the structure and just missed being the discoverer of the membranous cochlea.

The significance of the spiral tube seen by Huschke in the embryo and its persistence as the ductus cochlearis in the adult remained to be pointed out by Reissner and Reichert in a series of communications published in the years 1851 to 1854, being based in large part on embryological studies of the chick and also of mammals. The first communication was the Dorpat dissertation of the former, completed in Reichert's laboratory. It contained an account of the ductus cochlearis (canalis cochlearis), which was shown to exist as a definite canal in the adult mammalian cochlea. It was pointed out that the membranous part of the lamina spiralis forms one-half of the wall of the canal and that the other half consists of a very thin and delicate membrane that is usually torn in the preparation of such a specimen. This portion has since been known as the membrane of Reissner.

Reissner and Reichert demonstrated the complete canal in infants at about term. In the embryo they found that the cochlear duct opens into the membranous vestibule, but whether or not it does this in the adult was not definitely determined. They give an account of the development of the ear in the chick, and they describe the formation of a labyrinthine groove in the skin and how this subsequently invaginates to become the otic vesicle to which the acoustic nerve attaches itself later. They divide the early labyrinth into three chief parts: (1) recessus labyrinthi; (2) vestibule and its three canals; (3) cochlear duct. In a later paper Reissner (1854) refers to the formation of the scalæ. He explains them as two accessory cavities that are formed because the cartilage recedes from the upper and lower surfaces of the cochlear duct. He denies the existence of any communication between the vestibular cistern (Höhle des Vorhofs) and the scala vestibuli both in the embryo and adult. His observations concerning the scalæ have proved to be less important than those on the embryology of the otic vesicle and the discovery of the cochlear duct.

Kölliker (1854) had just at this time written the first edition of his "Gewebelehre" and had described at some length the finer structure of the cochlea, embodying his own and the important observations of Corti (1851). He makes mention of Reissner's dissertation, but there is no evidence that he appreciated at that time the significance of the cochlear duct. In a separate paper and in his text-book on human embryology, both of which appeared soon after this (Kölliker, 1861 *a* and *b*), he definitely establishes the existence of Reissner's membrane and that it forms the boundary of the cochlear canal. He confirmed the Reissner and Reichert embryological studies showing that the cochlear canal is originally an epithelial tube which is derived from the primitive ear-vesicle and hence from the ectoderm of the embryo. He designates the cochlear canal as the scala media, a term which persisted for many years, though its inapplicability was promptly pointed out by Reichert (1864).

Kölliker is the first to describe in some detail the formation of the otic capsule and the perilymphatic spaces. The summary here given is taken from the second edition of his book (Kölliker, 1879), in which there is some amplification of the account given in the first edition. According to him, after the otic vesicle reaches a certain degree of development it is surrounded by a delicate connective-tissue membrane and there is an outer thicker and firmer mass which takes on the nature of cartilage, which in 19-mm. cow embryos forms an integral part of the wall of the skull. In human embryos 8 weeks old the labyrinth capsule consists of true cartilage and completely fuses with the base of the skull. He expresses the opinion that the cartilago petrosa is laid down exactly in the same way as the other parts of the lateral walls of the skull and that its special characteristics, subsequently assumed, are due to the presence of the special sense-organ.

In connection with the origin of the cavities of the bony labyrinth, Kölliker draws attention to the problem of space formation in general and points out that the space in the otic capsule is of the type seen in the subarachnoid and other serous cavities. He describes how, along with the growth of the epithelial part of the labyrinth, there is also a rapid growth of its connective-tissue coverings, which soon attain considerable thickness. At the same time the periotic tissue becomes differentiated into three layers, of which the middle one soon becomes the thickest. This layer consists of a network of anastomosing connective-tissue cells (Gallertgewebe), whose rounded meshes are filled with fluid. From this there is gradually formed the cavity that surrounds the semicircular canals, the meshes becoming larger and finally coalescing. In the process of coalescence, parts of the cellular net are broken and other parts are pressed against the walls of the space, where, even in the adult, one can recognize remnants of the broken net. The same process takes place in the semicircular canals, the vestibule, and the cochlea. In the last-mentioned there are formed the two scalæ, in which, in addition to the coalescence of the spaces of the network, there is also involved a disproportionate growth in respect to the cochlear duct and the surrounding cartilage, the latter retracting from the former by virtue of its rapid growth. Kölliker's conception of this process is purely mechanical, and it hypothesizes a protoplasmic network that is entirely passive. He did not conceive of an adaptive activity on the part of the protoplasm itself by virtue of which the characteristic changes in form are brought about, as is to be described in the present paper.

Among a series of miscellaneous notes concerning the development of the mammalian labyrinth appended at the end of Kölliker's chapter, there is a reference as to the relation of the growth of the cartilage to the growth of the contained spaces, which is particularly interesting, as it shows that he had in mind one aspect of the problem with which we are concerned. He notes there (p. 746) that at first the epithelial part is directly surrounded by young cartilage, or better, a cartilage-like substance of which the greater part becomes subsequently converted into cartilage. In its further differentiation the tissue lying directly against the labyrinth becomes fibrous tissue and the tissue farther away becomes cartilage. Out of the uniform fibrous tissue there is further differentiated the inner perichondrium, the fiber wall of

the labyrinth, and the cell tissue intervening between these. This intervening tissue undergoes an independent growth characterized by a rich growth of blood-vessels. As a space, *i. e.*, the cavity of the cartilago petrosa, it grows in correspondence to the growth of the epithelial labyrinth. As to the behavior of the cartilage during the growth Kölliker was undecided, giving the opinion, however, that it grew independently at the same time as the space, and was not simply mechanically stretched out.

The embryological studies of His began to appear at about the time of the publication of Kölliker's work on the ear, and one would rather expect that the attention of this keen observer would have been attracted to this subject. In his "Akademische Programme" of the year 1865 he outlines (His, 1903) the general problem of the formation of the various body-cavities and describes in detail the formation of the cavities of the middle germ-layer. He includes in this the arachnoid spaces and the cavities of the eye-ball, but he does not refer to the ear.

Inasmuch as the present paper is directly concerned only with the capsule of the ear and the contained periotic connective-tissue spaces, it will not be necessary to trace the further elaboration of a more precise knowledge of the structure of the membranous labyrinth which rapidly took place following the appearance of Kölliker's text-book and the introduction of the new histological and embryological methods which were devised in such abundance at about that time. A complete survey of such investigations is given in the monograph of Retzius (1884), to which the reader is referred. Our review here of the subsequent literature will be restricted to those publications having a special bearing on the periotic connective-tissue structures and the problem of their development.

The canalis reuniens was discovered by Hensen (1863) as a communication existing between the ductus cochlearis and the sacculus. This established the relation of the cochlear duct as a definite part of the closed system of the membranous labyrinth, and its complete separation from the vestibular space. Using the terminology of Breschet, it thus constitutes an endolymphatic space, whereas the scala vestibuli and scala tympani are both perilymphatic spaces. Hensen also described the aqueductus cochleæ. He regarded it as an invagination from the outer perichondrium into the cochlea by a process similar to the invagination of the aqueous humor of the eye. In the embryo it consists of a connective-tissue tube which is continuous with the primary periosteum. It splits into two limbs, the shorter one of which extends towards the round window and forms the lining of the proximal part of the scala tympani. The other limb of the membranous aqueduct extends towards the modiolus and unites with the dura mater of the acoustic nerve.

Hensen was followed by Odenius (1867) who gives a careful description of the position of the different parts of the membranous labyrinth and of the "perilymphatic" spaces surrounding them. He separates the perilymphatic space of the vestibule into two divisions based on the attachment of the utricule to the vestibular wall. The lower and chief division he names sinus perilymphaticus vestibuli. This communicates with the upper division, which surrounds the upper part of the utricule and extends along the semicircular canals. This part is narrower and is hardly

more than a cleft situated between the membranous labyrinth and the bony wall and is traversed by many trabeculae.

A third author who could be put in this group is Boettcher (1869). He published two papers which bear upon the periotic spaces, but these are not available to the writer and resort has been had to the account of them given by Retzius (1884). He describes the formation of the scalae in sheep embryos. They make their first appearance in embryos 70 mm. long, beginning in the first turn of the cochlea and gradually extending to the second and third. According to him there is a preliminary formation of mucus tissue in the region in which the scale are to appear; this then undergoes a fatty degeneration, the result being the formation of the spaces. He warns against confusing this special "Schleimgewebe" with ordinary intracapsular connective tissue and opposes Hensen's theory of its invagination from the outer periosteum. According to him (Boettcher, 1872), it arises *in loco* out of the original cellular embryonal connective tissue.

Although it was recognized that there must be a provision in the human adult ear for the renewal and drainage of the intralabyrinthine fluid, yet there was no positive evidence of how this was accomplished until the introduction of injection methods. Schwalbe (1869) found that when Berlin blue is injected into the subdural space the injection mass passes through the internal auditory meatus into the space existing between the bony and membranous labyrinth. Since he could also trace the injection mass from the subdural spaces into the lymph vessels and glands, he therefore believed that the perilymph spaces represented true lymph spaces, for which the arachnoid spaces acted as the main drainage-channels.

In order to test out the communication reported by Schwalbe between the arachnoid spaces and the perilymphatic space, a series of injections were made by Weber (1869), who found that the injected fluid accompanied the acoustic nerve as far as the lamina cribrosa, but did not go through this. It passed rapidly, however, through the aquæductus cochleæ into the perilymphatic space of the cochlea. Later, this same investigator (Weber-Liel, 1879) invented the aspiration method by which the results were refined, and he was able to avoid the production of artificial paths which commonly result where strong pressure is necessary for the injection and which apparently had vitiated Schwalbe's experiments. He proved clearly that the aquæductus cochleæ was the primary path of communication between the perilymphatic and arachnoid spaces, and that it consists of a free canal lined by an extension of dura mater connecting the scala tympani with the cranial cavity. He was not specific, however, as to whether the communication was with the subdural or subarachnoid space.

Key and Retzius (1875) in their extensive studies on the brain membranes, were able by injection methods to show that the spaces of the brain membranes stood in open communication with the perilymphatic space of the labyrinth, but, although they were able to trace the injection mass along the acoustic nerve, through the lamina cribrosa, and along the finer filaments of the nerve into the lamina spiralis, they were not sure of its communication there with the perilymphatic space. They showed, on the other hand, that the latter could not be injected through the

aqueductus vestibuli and seemed convinced that the main communication was through the aqueductus cochleæ as described by Weber, which view remains the prevalent one to-day.

It may be added that Retzius (1884) subsequently made some further injection experiments in older fetuses and in the adult, which were published in his large monograph on the ear. He found (p. 330) that in this way the scala tympani communicates freely through the ductus perilymphaticus with the subarachnoid spaces, and not with the subdural space, which point had been left undecided by Weber. By injecting through either the round or oval window he was able to trace the escape of the fluid into the subarachnoid spaces, but never into the subdural space.

The comparative anatomists gave relatively little attention to the connective-tissue spaces around the ear and there was consequently no great advance secured from this aspect of the problem. Hasse (1873, account taken from Retzius) investigated embryos of various mammals, but his results are confusing. Concerning the lymph tracts of the inner ear he showed that in man and other mammals, in embryonal and adult stages, there exists a channel to which he gave the name ductus perilymphaticus, which is the same channel through which the injectionists had forced their fluids into the scala tympani. Hasse described this as provided with a sac which connects on the one hand with the cavum subaracnoideale (the outer epicerebral space after splitting the brain membranes into pia and arachnoid) and into a lymph-vessel on the other. This drainage path of the "perilymph," according to him, is not the only and in fact is not the chief drainage path; a similar path, consisting of a funnel-shaped sheath of arachnoid, projects into the internal auditory meatus accompanying the acoustic and facial nerves. In a later paper, Hasse (1881) reverses the importance of these two channels and describes the perilymph as flowing chiefly through the ductus perilymphaticus into the peripheral lymph system in the region of the jugular foramen, the same channel also draining the cerebrospinal fluid of the subarachnoid cistern. There is also, according to him, some drainage from the subdural space through the internal auditory meatus.

Of more importance is the description of Retzius (1884). In his large monograph on the ear the gross and finer morphology of the periotic spaces and especially of the higher mammals, is described in greater detail and completeness than had previously been done. The comparative embryology of the spaces is referred to by Balfour (1885). He speaks of lymphatic spaces (p. 522) as forming in the mesoblast between the membranous labyrinth and the cartilage. These spaces are partially developed in *Sauropsida*, but become larger and more important in mammals, where they form the two scala and the space surrounding the utricule and semi-circular ducts. According to him the scala begin to develop at the basal end of the cochlea, the cavity of each being gradually carried forward toward the apex of the cochlear canal by a "progressive absorption of the mesoblast."

The descriptions of Retzius (1884) and of Kölliker (1861 *b*, 1884) and also the chapter in the sixth edition of the "Gewebelehre," rewritten by v. Ebner (1902), have had a prevailing influence on the present conception of the character and development of the tissues of the otic capsule. There should be mentioned with

these also the work of Krause (1901), who studied the development of these structures in a number of vertebrate forms. He finds that the first traces of the formation of a "perilymphatic space" occur some little time before the formation of the scala tympani, in the region lateral to the utricle and saccule. At this point the perilymphatic tissue becomes gradually fluidified and there arises between the lateral wall of the two sacs and the cartilaginous wall of the labyrinth a large perilymphatic space—the cisterna perilymphatica—to which the foramen ovale serves as a direct approach. From this cistern the space-formation spreads into the cartilaginous semicircular canals and simultaneously there begins the formation of the scalæ, resulting finally in a cavity system that incloses the entire membranous labyrinth. Reference is made by him to the ductus perilymphaticus of Hasse, which connects this system with the subarachnoid spaces. This duct opens at one end in the vestibular part of the scala tympani and in the jugular fossa at the other. Concerning its development, nothing further was known.

In studying the development of the otic capsule, one is led into the general question of the growth of hyaline cartilage, for which there is an extensive literature and which is beyond the scope of the present paper. For general papers on this subject the reader is referred to those of Retterer (1900), Mall (1902), and Bardeen (1910). Other papers that may be mentioned as dealing particularly with the histogenesis of the skull are those of Solger (1889) and Filatoff (1906). An experimental study by Lewis (1907) should be referred to, in which it is shown that the production of the cartilaginous capsule is dependent upon the presence of the epithelial vesicle and that a transplanted otic plate becomes surrounded by cartilage derived from the tissue of the host.

The gross morphology of the cartilaginous capsule of the ear has been described for several embryonic stages in connection with the cartilaginous skull as a whole, and mention may be made in this connection of the work of Gaupp (1906) and Terry (1917) on certain vertebrate forms and the papers of Levi (1900) and Macklin (1914) on the human embryo. The writer has also had the opportunity of studying a reconstruction of the otic capsule in a human embryo 21 mm. long made by Professor W. H. Lewis and one of a 43-mm. embryo made by Dr. Macklin, both of which were modeled in this laboratory and have not yet been published.

MATERIAL AND METHODS.

The observations recorded in this paper are made on human embryos and cover the period included between 4 mm. and 130 mm., crown-rump length, which is approximately equivalent to the period between the fourth and the sixteenth week of fetal life. The embryos were taken from the collection made by Professor Mall and that now belongs to the Department of Embryology of the Carnegie Institution of Washington. With two exceptions they had already been prepared in serial sections. In most of the stages the whole embryo is included in the sections, in some of the older ones the head alone is included, and in the two specimens that were especially prepared for this investigation the sections include only the region of the temporal bone. In the following table are listed the embryos that were found

particularly suitable for the purpose at hand. They are arranged in the apparent order of development. The measurements given are those under which they are listed in the catalogue of the collection, and they all signify crown-rump measurement. Where sections were photographed this is indicated and the slide number, followed by the row and number of section, is given.

Table of Specimens.

Crown-rump length, millimeters.	Catalogue No.	Direction of section and thickness in microns.	Section photographed.	State of development of the periotic tissues.	
4	588	Coronal... 15	6-6-7	Beginning condensation of periotic mesenchyme. Distinct condensation of periotic mesenchyme.	
9	721	Transverse. 15	5-2-1		
11 8	1121	Coronal... 40	6-3-4	Definite capsules of mesenchyme surrounding labyrinth. Membranous canals begin to separate off from main pouch.	
11	353	Coronal... 10	16-3-4		
13	485	Coronal... 40	10-1-3	Condensed mesenchyme becomes precartilag.	
12.5	317	Coronal... 20		
13.5	695	Transverse. 10		
16	406	Sagittal... 20		
15	719	Transverse. 40	3-7-3		
14	144	Sagittal... 40	4-1-3		
16	409	Transverse. 20	6-3-5		Differentiation of precartilag into permanent and temporary types.
17 2	424	Transverse. 50	3-4-4		
17	296	Coronal... 20		
17	576	Sagittal... 15		Distinct capsules forming around precartilag nuclei in some areas.
21	460	Transverse. 40	14-1-1		
23	966	Coronal... 40	30-3		
23	453	Sagittal... 20		
24	455	Transverse. 30		
26.4	1008	Sagittal... 40	10-2-2		
26	1199	Coronal... 40	39-1-3		
27	756a	Coronal... 50	47-2		
27	875	Sagittal... 40	Beginning formation of definite periotic reticulum around canals.	
30	86	Coronal... 50	46-2		
33	145	Sagittal... 50	7-1-3	Precartilag becomes true cartilage. Reticular area surrounds epithelial canals.	
33	211	Sagittal... 50	14-3		
35	199	Sagittal... 50	58-2	Beginning space-formation in vestibular region.	
37	1318	Coronal... 100	42-1		
37	972	Sagittal... 50	20-1		
39	362	Sagittal... 50	Dedifferentiation of true cartilage into precartilag in region of canals.	
43	886	Coronal... 100	42-3		
50	84	Transverse. 50	146-2	Rudimentary periotic cistern present and scala tympani can be recognized. Scala vestibuli can be recognized.	
46	95	Sagittal... 100	72-1		
50	224	Sagittal... 50	20-1	Rapid dedifferentiation of precartilag around canals into reticulum.	
50	184	Sagittal... 50	23-2		
52	148	Sagittal... 25, 100	33-2		
59	96	Sagittal... 100	12-2	Vascular network established around canals.	
59	267	Sagittal... 20	29-1-2		
73	1373	Transverse. 10	9-3-1		
89	172	Transverse. 100	191	Perichondrium and membrana propria present in their early form.	
85	1100 30	Transverse. 100	43-2		
139	1018	Transverse. 50	30-1		
				Beginning formation of periotic spaces in region of canals.	

In studying the development of the cartilaginous capsule and the histogenesis of the periotic reticulum it was found necessary to prepare enlarged photographs of the special regions studied. By having these all made on the same scale of

enlargement it was possible to follow from stage to stage the change in volume and in form of the constituent tissue masses. Some of the photographs are reproduced on Plates I and II. In drawing conclusions from such photographs account was taken of the fact that the technique of preparing the serial sections introduces an element of uncertainty in that some embryos in the process of embedding shrink more than others. This is particularly so in human embryos, where there is necessarily some difference in the freshness of the material at the time it is obtained. Furthermore, even in the same embryo some tissues are affected by the technique more than others. Due allowance was made for these factors.

In order to determine the form and relations of the periotic-tissue spaces, wax-plate models of the membranous labyrinth and the surrounding spaces were reconstructed after the Born method. Advantage was taken of the improvements in the method devised by Lewis (1915). The serial sections were photographed at a suitable enlargement on bromide paper. By means of a preliminary model of the membranous labyrinth the necessary reconstruction lines were established and transferred to the bromide prints. From these prints the membranous labyrinth and the periotic spaces were then traced on wax plates. After cutting out from the plates the areas corresponding to these structures the plates were piled and the resultant cavity was filled with plaster of paris. When the wax was finally melted off there remained a permanent plaster cast of the objects desired at a definite enlargement. Views of these models are shown on plate 4.

In outlining the periotic spaces it was found necessary to adopt an arbitrary rule as to how much should be included in the model. The smaller spaces of the reticulum surrounding the main cavities can be seen coalescing to form larger spaces, and these in turn coalesce with the main cavity as it advances into new territory. There is thus a considerable range in the size and completeness of the spaces in any one section. The main spaces and the larger adjacent ones that communicate with them are outlined by a membrane-like border. This characteristic was adopted as the guide in determining which spaces to admit into the model; only those possessing a more or less complete border of this kind were included.

DEVELOPMENT OF THE CARTILAGINOUS CAPSULE OF THE EAR.

When the present study was undertaken the writer's interest concerned more particularly the process of conversion of the periotic reticular tissue into the walled-off spaces that constitute the scala tympani, the scala vestibuli, and vestibular cistern. It was soon found, however, that this could not be satisfactorily treated without a consideration of the earlier history of this tissue and its relation to the surrounding cartilaginous capsule. Therefore, a preliminary survey was made of the earlier histogenetic processes of all the mesenchymal elements of the inner ear. The character of these processes will form the subject-matter of the first part of this paper. In brief, they include: (1) the original condensation of the mesenchyme around the otic vesicle; (2) the subsequent differentiation of the condensed mesenchyme into precartilage on the one hand and periotic reticular tissue on the other; (3) the differentiation of true cartilage and its manner of growth and alteration in

form. After considering these, we shall be prepared in the second part of this paper to take up the alterations in the periotic reticular tissue that lead to the formation of the periotic spaces.

CONDENSATION OF THE PERIOTIC MESENCHYME.

If one looks at the otic vesicle in a human embryo from 4 to 5 mm. long, just as the endolymphatic appendage is becoming constricted off from the remainder of the vesicle, it will be found that the mesodermal tissue surrounding it is about the same in its appearance as that in other regions. There is the brain-wall, the otic vesicle, the ganglion mass connecting them, a few blood-vessels, and the ectoderm; otherwise there is to be seen only a more or less uniform mesenchymal synectium lying between these structures. Close against the vesicle the nuclei are perhaps a little more numerous. This can be seen in figure 5, which is taken from an embryo 4 mm. long (Carnegie Collection, No. 588). The section passes through the otic vesicle in its longest diameter and shows dorsally the endolymphatic appendage as it appears at this time. Lateral to the otic vesicle is the primary head-vein. A network of capillary vessels is spreading over the brain-wall, not extending quite to the ventral median line. Along the median margin of this sheet of capillaries there forms a larger channel which gradually separates itself from the capillaries and takes part in the formation of the basilar artery, as has been described in the chick and pig by Sabin (1917). The mesenchymatous tissue is denser in some regions than in others. The nuclei are quite sparse ventral to the brain-wall near the median line, becoming perceptibly more numerous as we approach the ear-vesicle. This increase in the number of nuclei in the neighborhood of the vesicle marks the beginning of the mesodermal condensation that is to form the otic capsule. It is not yet possible, however, to outline a definite layer of these nuclei.

When embryos are examined that are a little older than this it is found that a condensation of the mesoderm around the otic vesicle can be clearly recognized. Such a stage is shown in figure 6, which is from a photograph of a section of a human embryo 9 mm. long (Carnegie Collection, No. 721). Under low magnifications it is apparent that the mesoderm in the region of the vesicle is denser than the adjoining mesoderm, and particularly so on the lateral and ventral surfaces of the vesicle. The condensation of the mesoderm is also beginning on the median surface of the vesicle, but the process there is somewhat slower. The endolymphatic appendage, however, is free from any surrounding condensation; the mesoderm appears to be unaffected by its presence. The section in figure 6 passes transverse to the long axis of the vesicle. A small portion of the brain-wall is shown that is slightly retracted from the surrounding mesenchyme. The area of condensed tissue surrounding the vesicle is thick enough to extend from the surface of the vesicle to about half the distance from the vesicle to the ectoderm.

When analyzed under higher magnifications, it is found that the compact appearance around the vesicle is due to several factors. As compared with the mesenchymal synectium of the adjoining parts, the nuclei here are slightly larger, are more numerous, and are closer together. The intervening protoplasmic syn-

cytium is also denser and possesses wider trabeculæ, with correspondingly smaller spaces between them. This condensed tissue abuts, on the one hand, directly against the epithelial wall of the vesicle and forms a limiting membrane, as can be seen in places where the epithelium is retracted through shrinkage changes. On the other hand, it is directly continuous with the general mesenchymal syncytium, the transition between the two, however, being quite abrupt, as can be seen on careful scrutiny.

In embryos between 11 and 13 mm. long, which is just before the first semi-circular duct is separated off from the main labyrinth by the apposition and absorption of the intervening labyrinthine wall, the condensation of the mesoderm has advanced in thickness and extent so that it forms a nearly complete capsule for the epithelial labyrinth. Such a stage is shown in figure 7, which is taken from a human embryo 11 mm. long (Carnegie Collection, No. 353). This capsule encasing the labyrinth is thicker and denser on the lateral and ventral surfaces of the labyrinth, including the ventral pouch that is to form the cochlea. It remains incomplete on the median surface in the region of the nerve terminations. This latter space is occupied by the rootlets of the acoustic nerve-complex which bridge the short distance between labyrinth and brain and which are invested by a rich plexus of blood-vessels. It is this area that eventually becomes the internal auditory meatus. Slightly more caudal, near the glossopharyngeal nerve, can also be made out a deficient portion of the capsule that corresponds to the fenestra cochleæ (rotunda) and the aquæductus cochleæ. A third opening through the capsule is brought about by the endolymphatic appendage. This does not become encased by the capsule, but emerges dorsally to lie between the brain membranes and the skull. At first this latter opening is one in common with the internal auditory meatus. It very soon becomes separated off by the growth of the condensed tissue around the neck of the endolymphatic appendage. In figure 7 the section passes through the long axis of the membranous labyrinth. Only the vestibular portion is shown with the endolymphatic appendage opening out of it. The section passes transverse to the thickened margins of the pouches that are to form the superior and lateral semicircular ducts.

Thus at this time there is completely formed a condensed area of embryonic connective tissue surrounding the labyrinth that corresponds closely in form to the cartilaginous capsule into which it is about to be converted. On examining it under higher magnification there is found very little, aside from the condensation, that distinguishes it as yet from ordinary embryonic connective tissue. The condensed appearance is due to several factors. In the first place, the nuclei are more numerous in a given area. They also tend to be larger and rounder. Furthermore, the protoplasmic syncytium between the nuclei is denser, consisting of more numerous and more branched trabeculæ. In an embryo 16 mm. long, which had been stained with iron hematoxylin and erythrosin (Carnegie Collection, No. 406) the trabeculæ between the nuclei appear granular. This appearance is due to the presence of minute nodes that are found along the trabeculæ and which are stained deeply by the erythrosin, and add to the density of the tissue. Similar nodes are

found in the same embryo in the ordinary mesenchyme in that neighborhood, but are less numerous. This condensed tissue differs in one respect quite definitely from ordinary mesenchyme, in that it is almost devoid of blood-vessels, excepting along its margins. To all appearances it abuts, as in younger specimens, directly against the epithelial wall of the labyrinth.

DIFFERENTIATION OF PRECARTILAGE.

The histogenetic changes which mark the beginning of the conversion of the condensed mesenchyme into a cartilage-like tissue make their first appearance just after the separation of the semicircular ducts from the main vestibular pouch. This occurs in embryos about 14 mm. long. In embryos about 30 mm. long the otic capsule has the appearance and gives the tinctorial reactions of true cartilage. Thus, in embryos between 14 mm. and 30 mm. long, the otic capsule consists of a tissue that is intermediate between a condensed embryonic connective tissue and cartilage, and this intermediate form is known as precartilage.

The appearance of the otic capsule just at the time the canals are forming is shown in figure 8, which is from an embryo 15 mm. long (Carnegie Collection, No. 719). The section passes horizontally through the labyrinth. A portion of the utricle is shown at the bottom of the photograph, and detached from it, above, is the superior semicircular duct. A streak extending from the duct to the utricle still persists. This streak represents the wall of the labyrinth that formerly occupied this place and is now absorbed close up to the inner margin of the duct. Surrounding the capsule is a plexus of blood-vessels.

On examination under higher magnifications it is found that the tissue forming the capsule at this time differs very little from the condensed mesenchyme which we have seen in the younger stages. The most noticeable difference is that the nuclei are beginning to stand more apart from each other. This can be seen by comparing figures 7 and 8. In the former the section is 10μ thick, in the latter the section is 40μ thick. In spite of being four times thicker, the section of the older specimen shows only about the same number of nuclei that are seen in the thinner and younger specimen in figure 7.

Between the nuclei there are numerous branching slender processes. The spaces between the processes are not as clear as the spaces in the adjoining subcutaneous connective tissue, but contain a homogeneous substance that stains very slightly with such a dye as alum cochineal. The accumulation of this substance is doubtless related to the spreading apart of the nuclei and to the alteration in the branching processes that begins to show at this time. In certain regions the processes between the nuclei become less branched. Larger ones become more prominent and the smaller ones begin to disappear. A common arrangement is to find two or more larger processes uniting to form a loop at the side or at one or both ends of the nucleus. This feature is characteristic of precartilage. There is very little tendency as yet to an accumulation of denser protoplasm around the nuclei.

In embryos about 16 and 17 mm. long the optic capsule takes on a definite pre-cartilaginous character. This stage is shown in figure 9, which is from a photograph of an embryo between 17 and 18 mm. long (Carnegie Collection, No. 144). The embryo is listed in the collection as 14 mm. long, which is its measurement on the slide. Instead of this we use here its estimated formalin measurement, so as to conform to the other embryos, whose measurements are all given as in formalin. The section passes sagittally through the labyrinth. Above is shown the posterior semicircular duct and just below the center is shown the caudal end of the lateral semicircular duct, at the point where it widens out to join the utricle. By this time the differentiation of the tissue has advanced far enough so that one can properly speak of an otic capsule that is readily distinguished from any other condensed connective tissue. The outlines of the capsule are everywhere distinct. It fuses in part with the cartilaginous skull and it is continuous with the stapes. Embedded in it is the epithelial labyrinth together with its ganglionated nerves. The capsule envelops them entirely, except at the nerve entrance which is to form the internal auditory meatus, also at a point in the region of the jugular fossa that is to become the fenestra cochleæ and at the opening through which the endolymphatic appendage emerges.

On comparing figure 9 with figure 8 it will be seen that in addition to an actual increase in size the otic capsule is less uniform in appearance at this older stage. There are areas of denser tissue, or, rather, areas of more deeply staining tissue, which extend as streaks through the capsule inclosing other areas of less deeply staining tissue. The areas of less deeply staining tissue are in the immediate neighborhood of the semicircular ducts, completely encircling them and abutting directly against the epithelial wall of the ducts, as in the previous stage.

On examination under high magnification we find that the tissue forming the otic capsule at this time (embryos 17 mm. long) has for the greater part been transformed into precartilage. Precartilage, as seen in fixed material that has been sectioned and stained by the usual methods, differs from condensed mesenchyme chiefly in the alteration in the network of branching processes that extend between the nuclei. In condensed mesenchyme these appear as a syncytium of delicate refractile processes. In precartilage some of these become more sharply marked and linear, and are looped together so as to inclose an irregular space near each nucleus; the others become very finely subdivided and eventually disappear. While these latter processes are disappearing the area in which they lie takes on a homogeneous appearance. It does not take the stain, but it is more opaque than the inclosed spaces around the nuclei. Thus, instead of a syncytium the precartilage tissue gives the appearance of cell-islands separated from each other by a homogeneous matrix.

Regarding the exact structure of this slightly opaque substance our material does not suffice to warrant an opinion. This question must be approached by special methods. I may add, however, that remnants of fibrillar processes are found embedded in this substance for some little time after the walling-off of the encapsulated spaces or cell-islands. Each cell-island consists of a nucleus encap-

sulated by a clear space that varies in size and shape and whose contour seems to be formed by the persisting processes of the original syncytium. At first the nucleus is accompanied by very little condensed protoplasm, but this gradually accumulates after the formation of the encapsulated spaces and constitutes a cell-body of endoplasm. The nuclei continue to divide after the encapsulation and they can be seen in all stages of the process. The space shares in the subdivision and for a time each daughter nucleus inherits its own share of the space. The encapsulated spaces, in an embryo 17 mm. long (Carnegie Collection, No. 576), which had been stained deeply with hematoxylin and eosin, contained a homogeneous substance that was tinged with eosin. The substance was collected around the nucleus and filled more than half of the space of the capsule; but clearly it was not protoplasm and was not to be confused with the endoplasmic cell-body which forms later. None of this substance was found in the matrix surrounding the capsules.

The embryos in the Carnegie Collection that, on account of the stain that was used and the thinness of the sections, show particularly well the process of the differentiation of the encapsulated spaces are as follows: No. 576, 17 mm.; No. 409, 16 mm.; No. 296, 17 mm.; No. 409, 18 mm.; No. 455, 24 mm.; and No. 453, 23 mm. The order in which they are given indicates their relative development. In all of them areas are found showing different stages in the differentiation. On comparing them one could come equally well to two different conclusions regarding the encapsulation of the nuclei and the differentiation of the matrix. One could either say that the mesenchymal syncytium during the precartilage period undergoes a fusion into a semi-solid, homogeneous, slightly opaque mass in which the fibrils disappear and which forms the precartilaginous matrix, while at the same time selected spaces of the original syncytium develop a sharp margin and become encapsulated, each containing its own nucleus, or, one could say that the substance composing the matrix is deposited in the meshes of the syncytium, replacing most of the fibrils and obliterating the spaces except those selected ones that are inclosed by persistent processes and are encapsulated with an adjoining nucleus. One can not, however, see much evidence for considering the encapsulated spaces as of vacuole formation. They are certainly not vacuoles of the endoplasm, for the endoplasm does not make its appearance until after the spaces have taken on their characteristic form.

DIFFERENTIATION OF CARTILAGE.

The transition from precartilage to cartilage is a gradual differentiation that takes place in the otic capsule of embryos between 25 and 30 mm. long. If one examines an embryo 30 mm. long, such as shown in figure 11, it will be seen on comparing it with younger stages that the main capsular mass has undergone a distinct maturation. This transition is marked by a considerable increase in the amount of matrix combined with a more complete encapsulation of the nuclei, or cartilage cells as we may now call them. As the matrix increases in amount it also changes in its chemical composition, so that it is now possible to stain it differentially.

This tinctorial reaction makes an arbitrary point at which it may be said that precartilage becomes cartilage.

All parts of the capsule do not take part in this process equally. It has already been mentioned that during the period of differentiation of the precartilage the tissue of the otic capsule loses its homogeneous character and some areas of it begin to appear more dense than others. Immediately surrounding the semicircular ducts is quite a wide area of precartilage that appears less dense, which in turn is inclosed by the main precartilaginous mass of the capsule whose nuclei give it the appearance of greater density. This can be seen very well in figure 10. When we come to embryos between 26 and 30 mm. long this contrast between the two varieties of precartilage becomes more sharply defined, though the relative compactness of the arrangement of the nuclei becomes reversed. The semicircular ducts are then everywhere encircled by an area of temporary precartilage that differs from the rest of the capsule and which is not to become true cartilage, but is to be hollowed out to form the cartilaginous canals. This process of hollowing out the cartilaginous spaces and replacing with reticular connective tissue the precartilage that originally filled them forms a very interesting feature in the development of the otic capsule, to which we will refer later.

The difference between temporary precartilage and true cartilage is shown clearly in figure 11. This section passes transversely through the lateral semicircular canal of an embryo 30 mm. long (Carnegie Collection, No. 86). An area of temporary precartilage surrounds the epithelial duct, forming a dark circular field outside of which is the more permanent capsular mass. Examination under higher powers shows that the temporary precartilage differs from the main mass in that the nuclei are arranged somewhat concentrically, and there is less space between them than exists in the latter, which is the reason for its darker appearance. Furthermore, whereas the temporary precartilage around the semicircular ducts retains the general histological features that were seen in the younger stages, the main capsular mass has matured into well-defined cartilage. A specimen of about this same age is shown in figure 13 (Carnegie Collection, No. 199, 35 mm. long). This specimen was stained only in hematoxylin, which emphasizes the matrix. In such a preparation the cartilaginous matrix is stained intensely blue, whereas the temporary precartilage around the semicircular ducts takes the stain only in its nuclei. The reverse picture is shown in figure 12, where the tissues show an intense nuclear stain. This is taken from an embryo of about the same age as that shown in figure 13. Here, on account of the nuclei and the intervening dense protoplasm, the temporary precartilage forms a dark mass around the semicircular duct. Figures 12 and 13 are like a positive and negative and approximately indicate the outlines of the eventual cartilaginous canal. The area of temporary precartilage gradually retracts towards the border of the more permanent cartilage, as we shall see in the later stages, and as it does so the space becomes occupied by a reticulum of connective tissue.

In passing from embryos 30 mm. long to older stages, such as shown in figures 12, 13, and 14, the tissues show some advance in the degree of their maturation.

Their intense stain-reaction causes the area of temporary precartilagc to stand out very conspicuously. On examining under higher powers the section shown in figure 12 (Carnegie Collection, No. 972, 37 mm. long), it is seen that the nuclei in the precartilagc area are somewhat more numerous and are more compactly arranged than in the same area in figure 11. The darker appearance as contrasted with the surrounding cartilage is also due partly to the fact that the compact mass of internuclear protoplasm is distinctly tinged by the acid stains, whereas in the surrounding permanent cartilage the matrix is nearly devoid of any color, having been decolorized by the differential stain.

In addition to the staining reaction there is now a marked difference in structure between the more permanent cartilage and the temporary precartilagc. The latter retains its precartilaginous character. Its more peripheral cells show a slight tendency to capsule-formation. A common form among these is an oblong nucleus with thickened elongated processes at the four corners, resembling the pronged egg-case of the shark, the spaces between the processes on each side of the nucleus being parts of the incomplete capsular space. These cells are arranged in circular lines parallel with the circumference of the canal. The transition into true cartilage is rather abrupt, and on advancing into this region one meets with a characteristic matrix, embedded in which are the completely encapsulated nuclei. The temporary precartilagc in its more central layers, near the reticulum, does not show any tendency towards encapsulation. Its nuclei are arranged in concentric layers with a small amount of compact protoplasm between them, resembling an early stage of fibrous connective tissue.

A layer of blood-vessels marks the junction of the temporary precartilagc with the reticulum surrounding the semicircular duct. This reticulum appears lighter than the surrounding precartilagc because of the free spaces between its slender trabeculae. Furthermore, the nuclei are not quite so numerous and are more irregularly arranged. The reticulum does not advance very rapidly in its development, and it is not until we come to embryos between 40 and 50 mm. long that we meet with an extensive reticulum. The development of this reticulum will be described after we have taken up some of the subsequent changes in the cartilage.

GROWTH AND ALTERATION OF FORM OF THE CARTILAGINOUS CANALS.

In embryos 30 mm. long the cartilaginous labyrinth has attained approximately the adult form. Its subsequent development is primarily an increase in size to accommodate the growing membranous labyrinth. If a cast of the superior cartilaginous canal of an 80 mm. embryo be compared with a similar cast of the same canal in a 30 mm. embryo, it will be seen that the general form of the canal in the older specimen is much the same as in the younger specimen. But its diameter and length have both increased, the diameter being nearly doubled and the length trebled; furthermore, its linear curvature corresponds to an arc with a considerably longer radius. In reality, therefore, the developing cartilaginous labyrinth is continually undergoing changes, both in size and form. The histological evidence of these changes constitutes one of the most interesting features in the development of

this region, and although it does not directly concern the development of the contained periotic spaces, yet it may not be out of place to point out some of the elements of this process as they are seen in our material. In fact, the cartilaginous capsule of the ear is an especially favorable place for studying the general question of growth of cartilage, for two reasons: (1) there are, on account of the intricacy of form of the labyrinth, many kinds of cartilaginous changes found there that are necessary to accommodate its growth, including the deposit of new tissue and the removal of old tissues; (2) the topography is so well marked by known landmarks that all of these changes as well as the location and direction of growth can be easily followed:

Growth of cartilage is usually considered to be of two kinds, which are distinguished from each other by being either interstitial or perichondrial. Interstitial growth is described as consisting of an increase in the amount of hyaline matrix and the growth and proliferation of the encapsulated cartilage cells. The new cells form new capsules to a certain extent, but a point is finally reached beyond which the newly proliferated cells continue to occupy their parent capsule. From this variety of growth there results a uniform intumescence of the tissue without producing any marked change in its form. This manner of growth forms a large element in the increase in size of some parts of the capsule of the ear. In those parts, however, where a change in form is involved the growth is more like that described under perichondrial growth and consists of a new deposit of cartilage along the borders of the older cartilage, the constituent cells passing through a pre-cartilage stage. In the otic capsule this latter type of growth is actively going on even before a definite perichondrium is established. The deposit of new cartilage along the margin of older cartilage and the removal of old cartilage by dedifferentiation are indeed the main factors in the process through which the form of the ear-capsule is modeled.

The excavation of established cartilage can be studied by comparing sections through the semicircular canals at different stages, such as appear in figures 11, 12, 14, and 15. These are all sections through the same canal (lateral), taken in about the same position, and are enlarged the same number of diameters. It is, of course, possible that they were shrunken in different degrees in the process of embedding; this discrepancy, however, is probably not enough to interfere with their showing the approximate increase in size of the cartilaginous canal at the respective ages. A crude measurement of the perimeters of the canals as seen in the original photographs (100 diameters) yields the following circumferences: 30-mm. embryo, 115 mm. circumference; 37-mm. embryo, 132 mm. circumference; 43-mm. embryo, 152 mm. circumference; 50-mm. embryo, 192 mm. circumference. It is evident that we are dealing with an enlarging space and that a study of its receding edge must give the histological picture of the replacement of true cartilage by other tissue, either by dedifferentiation or by direct metaplasia.

If, with this process in mind, one makes an examination of the specimen shown in figure 11 (Carnegie Collection, No. 86) it will be seen under higher magnification that a rather definite border can be made out separating the general mass of true

cartilage from the inner zone of temporary precartilage surrounding the semi-circular ducts. The true cartilage has developed a considerable amount of matrix separating the encapsulated nuclei or cartilage cells. The margins of the capsules stand out as sharp refractive lines. The matrix lying between the capsules is slightly opaque and is beginning to take a differential stain. A narrow intermediate or transition zone separates the true cartilage from the precartilage; this zone is characterized by the presence of flattened and partially collapsed capsules between which there is very little or no matrix. The refractive margins of these overlapping, incomplete capsules give the appearance of wavy lines that run parallel with the margin of the canal. The same appearance is not seen in other regions of the otic capsule in younger stages, where precartilage is differentiating into cartilage.

In the process of cartilage differentiation in most parts of the otic capsule there is considerable intercapsular material at the time the margins of the capsules become conspicuous. The capsules are separated by the matrix-forming syncytium. Thus, there are not the conspicuous wavy lines due to overlapping capsules, such as characterize the intermediate zone. The transition between this zone and the true cartilage on one hand and the temporary precartilage on the other is quite abrupt in both instances. On entering the zone of precartilage there is found between the nuclei, instead of the wavy refractive capsular lines, a framework having more the character of a granular syncytium, with only here and there the suggestion of a beginning capsule. This, it will be remembered, is a condition the true cartilage exhibited in its earlier period. It is the intermediate zone to which we should address our especial attention, and it is this zone that moves outward as the cartilaginous canal widens.

Text-figure 1 shows a section through these zones in a fetus of about the same age as the one just described. This section is taken through the lateral

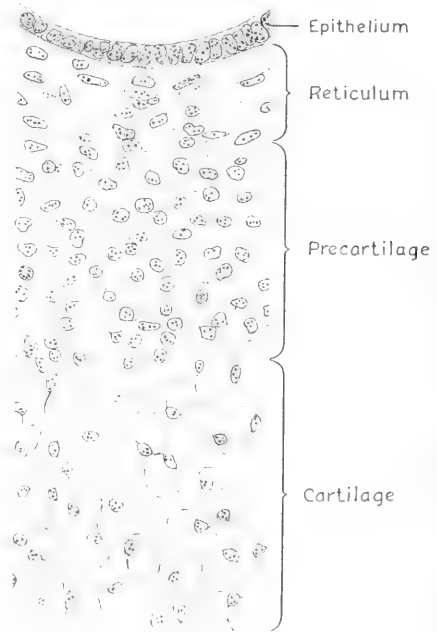


FIG. 1.—Detail of a section through the lateral semicircular canal in a human fetus 33 mm. long (Carnegie Collection, No. 145, slide 7, row 1, section 3). The section is 50μ thick and is enlarged 376 diameters. It shows the epithelial wall of the semicircular duct and the tissue zones that intervene between it and the cartilaginous capsule. In the outer part of the pre-cartilage zone is an intermediate area which is a transitional form between cartilage and precartilage. It is characterized by the scant amount of matrix and the incomplete and flattened condition of the capsule.

canal of a fetus 33 mm. crown-rump
The intermediate zone stands out con-

spicuously at the junction of the cartilage with the precartilage. Its wavy refractive lines are so compact that under low powers the whole zone appears as a dark rim outlining the cartilaginous margin of the canal. The compactness of these lines varies in different embryos of about the same age and even varies in the two borders of a given canal.

This latter condition can be seen in figure 14, which represents the lateral semi-circular canal of a fetus 43 mm. crown-rump length (Carnegie Collection, No. 886). It will be noted that the peripheral two-thirds of the intermediate zone (toward the right hand) forms a dark, heavy margin between the true cartilage and the encircled precartilage, whereas the central one-third (toward the left hand) is wider and much less distinct. It can also be seen that the place at which this intermediate zone is well marked corresponds to the direction of the excavation necessary to allow for the growth of the canal and to make room for the elongating semicircular ducts of the contained membranous labyrinth. In this case the expansion must be toward the periphery of the cartilaginous capsule, *i.e.*, toward the right side of the photograph. From studying various fetuses it seems to be true that where excavation of cartilage is actively going on at such a place there is found a prominent intermediate zone along the inner margin of the cartilage. Sometimes the line is uniform around the entire rim, but usually it is more marked on one side of the canal than on the other, and in such cases it is always toward the direction of the excavation of the cartilage, as can be judged from the topography of the labyrinth.

If an older specimen is examined, such as the one represented in figure 15, the character and relative position of the cartilage and precartilage are found to be the same as in the 30-mm. stage just described. They have, however, undergone an alteration to allow for the enlargement of the cartilaginous canal. Figure 15 shows the lateral canal in a human fetus about 50 mm. crown-rump length (Carnegie Collection, No. 95). The fetus is catalogued as being 46 mm. long, but this is apparently the slide-measurement. In its development it corresponds to fetuses 50 mm. long, formalin measurement, and this measurement is used so that it will accord with the other fetuses. Since figures 11 and 15 represent sections through the same canal taken at about the same place and under the same enlargement, one can superimpose them, one upon the other, and thus determine the change that has occurred between the two stages. If this is done it will be seen that the area that was precartilage in the 30-mm. stage is replaced by reticulum in the 50-mm. stage. There is just as much or more precartilage in the latter, but it has moved outward into the area that was previously true cartilage. In other words, the enlargement of the cartilaginous canal has been obtained by a process of excavation based on the dedifferentiation of true cartilage into precartilage and the latter in turn into reticulum. This is shown under higher magnifications in text-figures 2 and 3, which show sections of these same canals under the same enlargement and placed side by side for the purpose of better comparison. It can be seen in these two figures how the cartilage of 30-mm. stage becomes dedifferentiated into the precartilage of the 50-mm. stage and the border along which this process is in active operation forms the intermediate zone, which is characterized by its wavy, refractile

lines. The precartilage in turn is gradually dedifferentiated into the periotic reticulum. In this way the margin of the true cartilage gradually recedes from the epithelial duct, and the last of the precartilage is eventually dedifferentiated into a reticulum.

Along with the process of excavation of cartilage there must go the laying-down of new cartilage. For instance, as the lateral cartilaginous canal enlarges it also moves laterally, so that the distance between it and the carti-

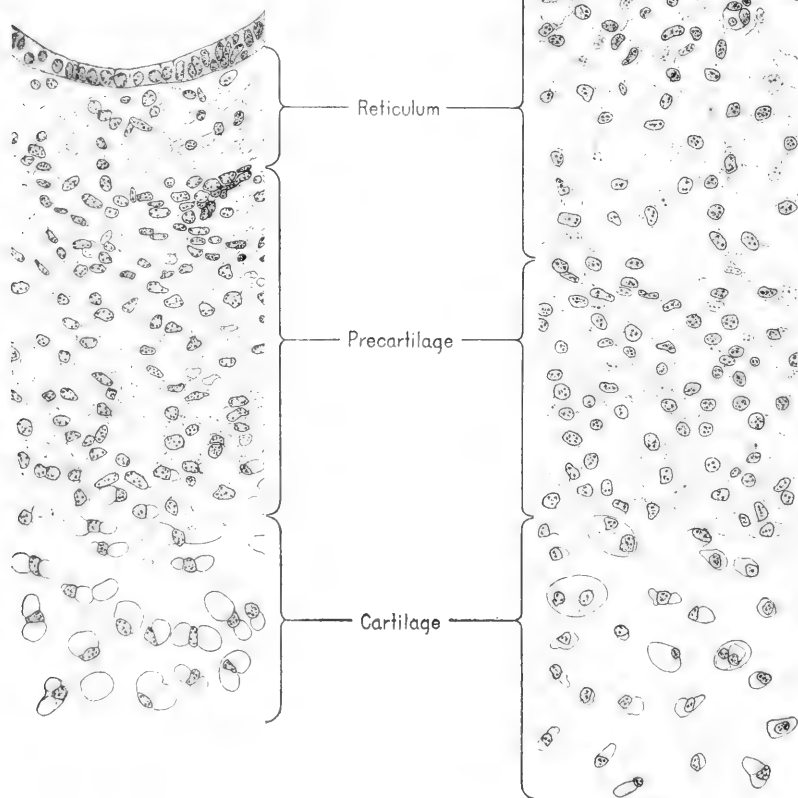


FIGURE 2.

FIGURE 3.

- FIG. 2. Detail of the lateral semicircular duct in a human fetus 30 mm. long (Carnegie Collection, No. 86), being the same as that shown in figure 11. The section is 50μ thick and is enlarged 470 diameters. It shows the epithelial wall of the duct and the character of the surrounding tissues that lie between it and the cartilage.
- FIG. 3. Detail of the lateral canal in a human fetus 46 mm. long (Carnegie Collection, No. 95, slide 72, section 1). This is the same canal as that shown in figure 15. The section is 100μ thick and is enlarged 470 diameters. By comparing it with figure 2 one can see how the cartilaginous canal is enlarged by the dedifferentiation of cartilage into precartilage and the precartilage in turn into the periotic reticulum.

luginous vestibule increases, producing relatively a lateral migration of the space as a whole. Such a migration involves the excavation of the established cartilage on its lateral margin and the formation of new cartilage on its median margin. On its lateral margin true cartilage is being dedifferentiated into precartilage and on its median margin precartilage is differentiating into cartilage. It is this phenomenon that determines the conditions shown in figure 14. On the right can be seen the prominent intermediate zone, indicating an active excavation of cartilage, and on the left the line of transition between cartilage and precartilage presents the same picture as that seen in the stage of differentiation of the latter into the former. One is forced to conclude that the cartilaginous tissue of the otic capsule is capable of differentiation and dedifferentiation in its earlier stages, at least up to the time of the completion of the encapsulation of the cartilage cells. This progressive and retrogressive adaptability of the cartilaginous tissue makes possible the changes that are necessary in the growth and alteration in form of the labyrinth.

DEVELOPMENT OF PERIOTIC RETICULAR CONNECTIVE TISSUE.

The formation of the connective-tissue reticulum surrounding the semicircular ducts is first indicated by a cluster of darkly stained nuclei that lie along the central edge of the ducts in embryos soon after the ducts are formed and before the differentiation of the cartilage is completed. In figure 9 such a cluster is seen just under the posterior duct in the upper part of the photograph. In figure 10, which shows the lateral semicircular duct of an embryo 27 mm. long (Carnegie Collection, No. 756*a*), a similar cluster of nuclei can be seen just under the duct, in reality just median to it. These foci mark the points at which the formation of the reticulum begins. It is not, however, until we come to embryos about 30 mm. long that we find a definite reticulum. At that time, as is shown in figure 11, a narrow lighter area can be made out, situated between the epithelial wall of the duct and the temporary precartilage. It is the development of this area at the expense of the temporary precartilage that results in the reticulum in which the periotic spaces are subsequently formed. This area consists of a mesenchymal syncytium containing irregularly shaped clear tissue spaces and is characterized by the presence of numerous blood-vessels and connecting capillaries. The larger vessels are found resting against the inner margin of the temporary precartilage. They sometimes indent it, but never penetrate it to any extent. Such vessels can be seen in figures 11 and 12. The presence of these blood-vessels is coincident with the appearance of the reticular tissue.

In describing younger stages the statement has been made that the temporary precartilage abuts directly against the epithelial wall of the semicircular duct. This statement is based only on the gross appearance. On careful scrutiny of the tissue that immediately surrounds the ducts in embryos between 14 mm. and 20 mm. long a few mesenchymal cells can be found which possibly do not belong to the temporary precartilage. These cells may very well represent some of the indifferent mesenchyme, and possibly also some angioblasts. It is conceivable that these surround the otic vesicle in its earliest stages and are inclosed along with

the otic vesicle by the condensed tissue of the otic capsule, where they remain in contact with the epithelial labyrinth in a resting condition until the embryo approaches 20 mm. in length. They then show activity and by the time the embryo is 30 mm. long we find them converted into a vascularized reticulum which forms a definite area surrounding each semicircular duct and completely separating it from the receding precartilage. The area of reticulum advances as the precartilage becomes hollowed out. This can be seen by comparing figures 11, 12, 14, 15, and 16, all of which are reproduced on the same scale of enlargement.

From the histological appearance one could maintain that the reticulum is derived from a few predestined mesenchymatous cells which, after a latent period, undergo proliferation and occupy the space that is vacated by the receding precartilage in the manner described above, the growth of the reticulum perhaps being the cause of the recession of the cartilage. But one could equally well maintain that the reticulum is derived entirely from the precartilage; that it is not a pre-determined tissue, but simply precartilage that has undergone dedifferentiation. It is entirely possible that the isolated cells included with the epithelial labyrinth are angioblasts only, everything else being indifferent mesenchyme. In the early stages, where only a few cells are concerned, this matter can not be determined, the histological difference between early precartilage and other embryonic cells not being sufficiently great for their certain recognition. In the later stages, however, it is quite evident that precartilage tissue is actually converted into a reticulum; that the replacement of the temporary precartilage by a reticular connective tissue is accomplished by a process of dedifferentiation, or direct metaplasia, just as we have previously seen in the case of the dedifferentiation of cartilage into precartilage.

In this connection it is instructive to compare again figures 11 and 15, and also figures 2 and 3, which are details of the same under higher magnification. They show under the same enlargement a section through the lateral canal made in about the same position and cut at the same thickness. It will be noticed that the space occupied by precartilage in the younger stage is entirely filled in by reticulum in the older stage. There is in the older stage, however, more precartilage than before, but it now occupies a more peripheral position. With the change in the position of the precartilage area there is a corresponding enlargement of the lumen of the true cartilage, *i. e.*, the cartilaginous canal. It is clear that we are dealing here with a dedifferentiation of true cartilage into precartilage on the one hand and a dedifferentiation of precartilage into reticulum on the other. These factors, as we already have seen, are of great importance in the alteration in form and size of the cartilaginous canals.

In younger stages, as in figure 10, the epithelial semicircular duct lies near the center of the area of temporary precartilage. When the reticulum develops it makes its first appearance, and its growth continues more marked along the concave side of the duct than on the convex side—that is, on the side toward the utricle rather than toward the periphery of the capsule. On this account the epithelial duct loses its central position and gradually comes to lie along the peripheral border of the cartilaginous canal, where it eventually becomes attached to the periosteum.

This eccentric position gives the canal the largest arc that is possible in the space in which it lies. It marks the point of thrust of the elongating duct against the cartilaginous chamber that confines it and it is in this direction that the cartilage must be excavated to make room for the further growth of the duct.

The spread of the reticulum into the surrounding precartilage is rather slow at first. There is very little advance made in fetuses between 30 mm. and 43 mm. long, as can be seen by comparing figures 11 to 14. In figure 14 the reticulum can be recognized as a crescentic-shaped area on the central side (toward the left) and partially surrounding the epithelial duct. In the figure it is about 0.8 cm. wide at its widest point. The surrounding precartilage is also of about the same width, but it is uniformly wide around the whole circumference of the cartilaginous canal. In fetuses about 50 mm. long the dedifferentiation of precartilage into reticulum makes more rapid progress. The change is quite abrupt at this time. Figures 14, 15, 16, and 17 form a series in which is shown the alteration from a small amount of reticulum to an almost complete reticularization of the cartilaginous canal. These changes are found in fetuses varying from 43 mm. to 52 mm. long. In comparing these figures one would expect that the membranous duct would be found progressively larger in the series of photographs if they were correctly arranged in the order of their age. But it should be remembered that the tissues show different degrees of response to the fixing reagents. This is particularly so in respect to the epithelial duct; in figures 14 and 17 it is distended, as can be seen by its thin wall, while in 15 and 16 it is contracted. The order in which they are arranged corresponds to their relative age, as far as could be determined by the records of the fetuses and general appearances of the sections.

In figure 15 there is a zone of precartilage, about 0.8 cm. wide in the photograph, which in reality is true cartilage that has been dedifferentiated into precartilage. The reticulum extends from the inner border of this to the membranous duct. In figure 16, which is a section through the posterior canal of a fetus 50 mm. long (Carnegie Collection, No. 184), the dedifferentiation of precartilage into reticulum has occurred faster than that of cartilage into precartilage. There is practically none of the latter to be seen; the whole of the space between the margin on the cartilage and membranous semicircular duct is filled in by reticulum. Along the central margin of the duct there are still seen thick clusters of proliferating nuclei which are associated in part with the development of the blood-vessels and in part with the modification of the reticulum that takes place around the wall of the membranous duct.

It has been noted that precartilage is free of blood-vessels, whereas the reticulum is vascularized from the very first. Part of the dedifferentiation of precartilage into reticulum consists of the invasion of blood-vessels into the precartilage region. In the early stages of the reticulum the larger vessels hug closely against the precartilage and continue to do so as the latter recedes from the epithelial duct, as can be seen in figures 11, 12, and 14. Later, with the abrupt dedifferentiation of the remaining precartilage into reticulum, the larger vessels do not follow the receding margin of the cartilaginous canal, but form vascular arches that are

suspended in the reticulum, as can be seen in figures 15, 16, and 17, and from these a network of small vessels branches toward the membranous duct on the one hand and the cartilaginous wall on the other.

In figure 17, which is a section through the posterior semicircular canal in a fetus 52 mm. crown-rump length (Carnegie Collection, No. 96), the reticulum is more mature in its appearance than any that have thus far been described. There is practically no precartilage to be seen. The reticulum now only lacks the membrane-like thickening of its inner and outer margins to render it complete. At the inner margin the cells arrange themselves into a fibrous coat that constitutes the membrana propria of the membranous duct. At the outer margin is formed the perichondrium, the development of which will now be considered.

DEVELOPMENT OF THE PERICHONDRIUM.

In the description of the development of the periotic reticulum we have seen how it begins as a small focus along the central border of the epithelial semicircular duct and spreads at the expense of the temporary precartilage, forming as it does so a crescentic-shaped area of reticulum inclosing the duct. We have also seen how the invasion or spread of the reticulum into the surrounding area of precartilage is brought about, at least in the later stages, by a dedifferentiation of the latter into the former.

Furthermore, along with this latter process, the inner margin of cartilage surrounding the duct is dedifferentiated into precartilage, so that a new area of precartilage becomes established as the old area disappears. The conversion of precartilage into reticulum in the later stages, however, is more rapid than the conversion of cartilage into precartilage, and consequently there comes a time when the precartilage has nearly all disappeared. In such specimens the reticulum extends practically from the epithelial duct to the margin of the cartilaginous canal. The qualifying term "practically" is used because the inner and outer margins of the reticulum are modified in a special manner. The inner margin becomes condensed into a membrane-like coat of fibrous tissue that constitutes the membrana propria of the membranous canal. The outer margin at about this time undergoes changes that result in the formation of the perichondrium.

In discussing the perichondrium it is important to keep in mind the active alterations in the tissue along the margin of the cartilage that accompany the growth of the labyrinth. It has been seen how the enlargement of the cartilaginous canals and their alterations in form and position is obtained partly by excavation of cartilage and partly by the laying down of new cartilage, the excavation being accomplished by its dedifferentiation into precartilage and reticulum, and the new cartilage being built up through a precartilage stage from the periotic reticular tissue. Throughout the entire period of growth of the cartilaginous canals the elements of this continual transformation exist along their margin. The margin during this period is in a state of temporary equilibrium and is capable of advancing or receding as the conditions determine.

The first and relatively the major part of the hollowing-out of the cartilaginous canals is complete before the perichondrium makes its appearance. This is illustrated, for instance, by the fetus of 52 mm. crown-rump length, in figure 17, where there is as yet no indication of it shown. In fetuses between 40 and 50 mm. long the zone of precartilage surrounding the margins of the canals, as seen in figures 14 and 15, might be mistaken for perichondrium. This area, however, in fetuses slightly older is converted almost entirely into reticulum. Kölliker (1879), in the second edition of his text-book on embryology, pictures a transverse section through the lateral canal of a rabbit embryo (fig. 457, page 735), in which this zone of precartilage is labeled as periosteum of the future bone.

The real perichondrium does not make its appearance until the fetus reaches a length of about 70 mm. A specimen of this age is represented in text-figure 4, which shows a segment of the posterior semicircular canal in a fetus 73 mm. crown-rump length (Carnegie Collection, No. 1373). On examination of this specimen it is found that there is a distinct condensation of the reticulum along its inner margin, so that it forms a *membrana propria* for the epithelial duct with which it is in contact. This area has largely lost its reticular character and now resembles embryonic fibrous connective tissue. Along the outer margin of the reticulum a similar condensation of its trabeculae has taken place, forming a thin fibrous lamina or membrane near the margin of the cartilage. This is the perichondrium in its early form. It does not abut directly against the cartilage, but is separated from it by a thin layer of transition tissue that is in process of dedifferentiation from precartilage into reticulum.

Passing inward from the cartilage, the transitions are rapid from cartilage to precartilage, from precartilage to the tissue that is in transition to the reticulum and then to the perichondrium. These are found as narrow zones that merge quickly from one into the other. One should remember that the cartilaginous canal has not reached its full size yet, and that the margin of the canal is still in an unstable condition. However, as the canal becomes larger and the tissues more mature, it is found that the transitions between the different zones become more abrupt and in this process the precartilage zone becomes relatively much narrower. This can be seen by comparing text-figures 3 and 4. The width of the reticulum in these two figures can not be compared, because they represent different canals, lateral and posterior, and no attempt was made to take them from the same relative positions. The fact that the reticulum is narrower in figure 4 has no significance in the question of growth. The wide precartilage zone in figure 3 as compared with that in figure 4, on the contrary, has a direct bearing on the relative age of the two specimens. A relatively wide zone of precartilage is characteristic of younger stages. After fetuses become 70 mm. long the precartilage zone becomes quite narrow, so that the transition from cartilage to perichondrium is relatively abrupt. In older specimens one might easily obtain the impression that the perichondrium rested directly against the cartilage, as doubtless it does in the adult. In the oldest fetus examined, 130 mm. crown-rump length, there is still found a distinct though

narrow precartilaginous-reticular transitional zone between the cartilage and the perichondrium. Presumably this indicates that the margin is still in an unstable condition.

After the perichondrium has made its first appearance it rapidly becomes thicker and more conspicuous. In a fetus 80 mm. crown-rump length (Carnegie Collection, No. 172) it is found as quite a dense fibrous coat, more than twice as thick as that shown in the 73 mm. embryo in figure 4. It is clearly separated from the cartilage and precartilaginous by a narrow zone of reticular tissue.

The character of the perichondrium as existing in slightly older fetuses is shown in figure 18, which represents a section through the posterior semicircular canal of a fetus 85 mm. crown-rump length (Carnegie Collection, No. 1400-30). Here the perichondrium consists of a relatively broad zone of embryonic fibrous connective tissue, which in the photograph is about 5 mm. wide, encircling the whole canal. It can be seen on the median side (to the left) that it is separated from the cartilage and adjacent transforming precartilaginous zone by a narrow, lighter area, which under higher magnification is found to consist of reticular tissue. The membrana propria at the inner margin of the reticulum is fairly well developed and it can be seen how it forms a supporting coat to the epithelial duct.

When one examines the cartilaginous semicircular canals in fetuses 130 mm. long there can no longer be any question as to the identity of the perichondrium. A specimen showing the superior semicircular canal at this stage is represented in figure 19, which is taken from a fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018). The blood-vessels are injected with India ink. The main cartilaginous mass in this specimen is quite mature; the capsules are well defined and the cartilage cells now possess a considerable amount of granular body-protoplasm.

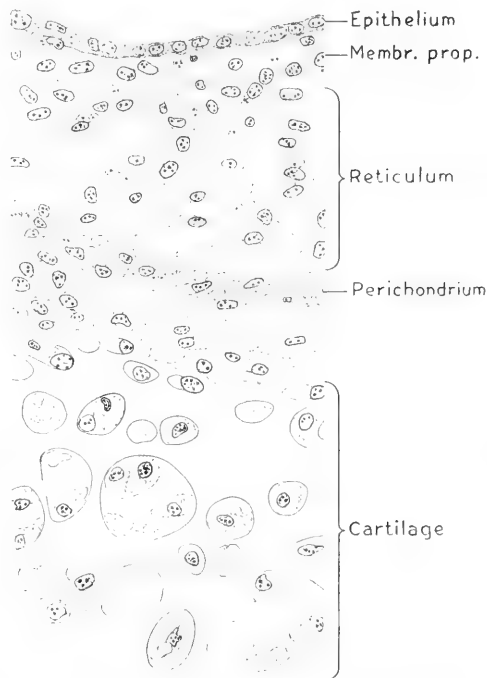


FIG. 4.—Detail of the posterior canal in a human fetus 73 mm. long (Carnegie Collection, No. 1373, slide 9, row 3, section 1). The section is 10 μ thick and is enlarged 375 diameters. It shows how the inner margin of the reticulum becomes condensed into the membrana propria of the epithelial duct and the outer margin into the perichondrium. The perichondrium does not lie in direct contact with the cartilage, but is separated by a narrow zone of tissue which consists of precartilaginous, into which the cartilage is still being dedifferentiated.

In many instances capsules are found containing more than one cartilage cell, showing the tendency to cell columns.

A casual glance at a section under lower powers might indicate that the inner margin of the cartilage is in direct contact with the perichondrium. Examination under higher magnification, however, shows that between the thick perichondrium and the cartilage there is a narrow zone of dedifferentiated cartilage. In it the matrix has largely disappeared and the capsules have collapsed and are flattened out, allowing the elongated endoplasm of adjacent cartilage cells to come in contact, separated only by the remnants of the capsular margins. Dyes that stain endoplasm red cause this zone to appear as a deep-red line. This zone represents a state of transition between cartilage and precartilage and its presence doubtless indicates that the margin of the cartilage is still in an unstable condition. The narrowness of the zone and the abruptness of the transition are characteristic of later stages, where the process is more gradual and relatively small in amount. The transition from this zone to the perichondrium is likewise abrupt. The perichondrium consists of a dense protoplasmic stratum thickly studded with nuclei, and has all the appearance of late embryonic fibrous connective tissue. It is of about the same thickness around the whole margin of the canal. At the outer margin (toward the right) it fuses with the membrana propria of the epithelial duct, thereby forming an attachment which is regarded as a suspensory ligament for the support of the membranous labyrinth. The trabeculae of the reticulum extending between the membrana propria and the perichondrium are just beginning to break apart, allowing the adjacent spaces of the reticulum, as they are seen in section, to coalesce in the formation of larger spaces.

Having completed the review of the early history of the reticulum and its formative relations to the adjacent tissues, we are now in a position to consider the development and the fate of these larger spaces in the reticulum, which have hitherto been generally known by the misleading term "perilymphatic spaces."

DEVELOPMENT OF PERIOTIC TISSUE—SPACES.

In the preceding pages of this article the main features of the development of the cartilaginous capsule that incloses the membranous labyrinth have been described. We have traced the process step by step from the first condensation of the mesenchyme around the otic vesicle, through its differentiation into a pre-cartilaginous mass and the maturation of the latter into true cartilage, with the formation through dedifferentiation of cartilaginous chambers in which the membranous labyrinth is suspended. It has been shown how these spaces within the cartilaginous capsule are modified in adaptation to the continued growth of the membranous labyrinth and how they finally come to be filled with an open-meshed reticulum which everywhere bridges the space existing between the membranous labyrinth and the surrounding cartilage. It has further been shown that the membrana propria supporting the epithelial part of the labyrinth on the one hand and the perichondrium on the other are derived from and serve as the limiting membranes of this reticulum. It is a modification in the meshes of this same reticulum

that results in the formation of the so-called perilymphatic spaces, or periotic spaces as they will be referred to in this paper, the development of which will now be outlined.

Thus far attention has been directed primarily to regions included in typical transverse sections through the semicircular canals. This was done for the purpose of uniformity and simplicity and because of the ease with which successive stages could be compared with one another. For studying the periotic spaces, however, the region of the canals is not so favorable, because the spaces are late in developing there, and even in their completed form they are not so well defined and highly differentiated as those in the region of the vestibule and cochlea.

The earliest evidence of a periotic space makes its appearance opposite the stapes. It is developed in the reticulum that fills the interval situated between the saccule, utricle, and the cartilaginous stapes. Even before the general periotic reticulum becomes very extensive, in embryos between 30 and 40 mm. long, it can be seen that its meshes are more irregular and more open in this region than elsewhere. This is the rudimentary form of the periotic vestibular cistern, which is the first space to become established.

DEVELOPMENT OF THE PERIOTIC CISTERNA OF THE VESTIBULE.

Aside from the scala vestibuli and the scala tympani, the largest of the periotic spaces is the large reservoir situated between the tympanic wall of the bony vestibule with its articulated stapes and the vestibular chambers of the membranous labyrinth. This is the spatium perilymphaticum vestibuli (BNA) or the cisterna perilymphatica (Retzius). In order to eliminate the word lymphatic from the terminology it will be designated here as the cisterna periotica vestibuli, or less formally the periotic cistern. In this manner the descriptive term introduced by Retzius is retained.

Before there is any trace of the scala the initial steps in the formation of the cistern can be seen. This is well illustrated in an embryo 35 mm. long (Carnegie Collection, No. 199). This particular embryo is cut in a sagittal series and the sections on slides 53 and 54 show the periotic cistern in its most rudimentary form. It consists of an area of reticulum bounded by the utricle, saccule, ductus reuniens, the proximal end of the cochlear duct, and the ampulla of the posterior semicircular duct. The greater part of the periotic reticulum at this time (35-mm embryo) is characterized by a narrow and uniform mesh that is interrupted only by numerous capillaries branching through it; in the area mentioned, however, the spaces are larger and are more irregular both in shape and in size. They present the appearance seen along the semicircular ducts in considerably older embryos, for instance, in the 52-mm. embryo, as is shown in figure 17. From the very first the increase in the size of the mesh seems to be attained by the detachment and retraction of its constituent protoplasmic bridges, thereby allowing adjacent spaces to unite in the formation of composite large spaces. Thus in the above section a few irregular protoplasmic free-ends are seen still projecting into the newly enlarged spaces. This interesting histogenetic process will be taken up again later in connection with

the development of the two scalæ. The area of this rudimentary periotic cistern is as yet very small and merges indefinitely into the adjoining reticulum. It is not until we come to fetuses about 40 mm. long that it develops spaces of any considerable size, and it is not until we come to fetuses about 50 mm. long that we find a single large space with walls that are definitely outlined, so that it can be satisfactorily modeled.

In a fetus 43 mm. long (Carnegie Collection, No. 886), which is cut in a coronal series, the spaces forming the rudimentary cistern stand out much more definitely than is the case in the 35-mm. embryo that has just been referred to. There is now just opposite the stapes one space which is much larger than the adjoining spaces. On part of its margin the protoplasmic bridges are stretched along so as to form a smoothly curved continuous boundary, which is defective in some portions, and at such places the space merges with the adjoining secondary spaces. Within the space are some faintly refractive branching threads of coagulated plasma. The scala vestibuli is not yet laid down and the scala tympani is only represented by a moderate widening of the meshes of the reticulum in the neighborhood of the fenestra cochleæ (rotunda), along the basal border of the first turn of the cochlear duct.

In fetuses 50 mm. long the outlines of the cistern become very distinct, due to the marked increase in the size of its main cavity and to the more definite membrane at its junction with the rest of the reticulum. Its form and relations are shown in figures 26 and 27. They represent a median and a lateral view of a wax-plate reconstruction of this region in a human fetus 50 mm. long (Carnegie Collection, No. 84). Only the main cavity is shown in the model. At certain places around its borders the meshes of the reticulum are uniting into larger spaces and these in turn are taken up by the main cavity as it advances into the new territory. These smaller incomplete spaces were omitted in constructing the plates of the model. The rule was adopted that only the spaces that were outlined by a membrane-like border should be traced on the plates and included in the model. This rule was adhered to in all the models of this series.

Figures 26 and 27 show that the periotic cistern in 50-mm. embryos consists of a flattened, rounded, bursa-like cavity intervening between the stapes and the lateral surface of the saccule and adjoining utricle. It extends forward to the ampulla of the lateral canal and upward to the beginning of the crus commune. Posteriorly it crowds backward against the ductus reuniens, filling in the space between the utricle, saccule, and the proximal end of the cochlear duct. Both on its median and lateral surfaces there is no further opportunity for expansion except as the vestibule itself enlarges. The delicate membrane-like wall of the cistern hugs closely against the parts of the membranous labyrinth on the one side and the tympanic wall of the cartilaginous vestibule on the other, being separated from them only by a thin layer of the original reticulum. Along the dorsal margin of the cistern, however, there is room for expansion, and the reticulum in this region shows enlarging spaces in the process of uniting with the main cavity. On its ventral margin, near the cochlea and extending along the apical surface of the latter, there

is a definite row of reticular spaces actively coalescing and constituting the beginning of the scala vestibuli. These are shown in figure 21, which is a section of a fetus of about the same age. The spaces of the scala vestibuli lie between the cochlear duct and the cistern. This section also shows very well the relation of the stapes to the cistern. The scala tympani is already well started at this time, but its development is quite independent of the cistern. Within the cistern can be seen scattered clumps of faintly refractive granular threads of what seems to be a coagulated constituent of the plasma.

The subsequent growth of the cistern is shown in figures 28 to 31. Figures 28 and 29 show respectively a median and lateral view of a wax-plate reconstruction of the membranous labyrinth and its periotic spaces in a human fetus 85 mm. long (Carnegie Collection, No. 1400-30). The growth of the cistern here has kept pace with the increase in size of the labyrinth and maintains the same general relations as regards the stapes and the parts of the membranous labyrinth. The view of the cistern in figure 28 is an oblique one which would tend to mislead one as to its width. In reality it is relatively a little wider. It has also extended upward on the dorsal surface of the utricle and is beginning to creep along the inner side of the posterior end of the lateral semicircular duct. Ventrally it communicates freely with the scala vestibuli, which now extends well down along the cochlear duct.

The oldest stage studied is shown in figures 30 and 31. These show two views of a wax-plate reconstruction of these structures in a human fetus 130 mm. long (Carnegie Collection, No. 1018). At this time the periotic cistern has spread over the vestibular part of the membranous labyrinth, covering it nearly everywhere excepting at the macular portions where the nerves terminate. In figure 31 it can be seen that the mesial surface of the saccule is not covered; this lies close against the wall of the cartilaginous vestibule. The uppermost division of the cistern, situated between the crus commune and the ampulla of the posterior semicircular duct, does not yet open into the general cavity. It has formed separately and owing to the position in which it lies its coalescence with the other parts of the cistern is retarded; otherwise, free communication exists between all divisions of the cistern.

DEVELOPMENT OF THE PERIOTIC SPACES OF THE SEMICIRCULAR DUCTS.

From the descriptions given of the adult the reticulum along the ducts never develops a single continuous wide periotic space like that of the cistern and the two scalæ. There always remain a few trabeculae, such as are seen in the cistern and scalæ in their earlier stages, and these constitute partitions which traverse the space and give it in sections the appearance of a series of separate spaces extending along the inner margins of the semicircular ducts. Although these spaces along the ducts are incomplete as compared with the cistern and scalæ, they are, however, entirely analogous with them in their formation.

The space along the lateral semicircular duct is the largest. Its posterior end exists as a continuation of the cistern. This can be seen in the lateral view of the model shown in figure 30, where the cistern extends for a considerable distance

along the inner border of the lateral duct. Along the other two ducts of the same specimen (130 mm. crown-rump length) the reticulum has commenced the process of space-formation, but complete channels are not yet established. A typical section through one of the semicircular ducts in a fetus of this size, and this is the oldest fetus studied, is shown in figure 23. As compared with the *scalæ* in the same fetus, as shown in figure 20, the space-formation along the ducts is very much retarded.

DEVELOPMENT OF SCALA TYMPANI AND SCALA VESTIBULI.

The *scala vestibuli* may be regarded as an extension of the cistern downward into the region of the cochlea and as such its growth starts from a focus opposite the *fenestra vestibuli* (*ovalis*). The *scala tympani* in a similar way makes its first appearance opposite the *fenestra cochleæ*. From these two foci the *scalæ* extend gradually downward along the cochlear duct as two separate spaces which do not communicate with each other until they reach the tip of the duct, where there is finally developed a free opening between them known as the *helicotrema*.

In their formation they go through a series of histogenetic changes essentially in the same manner that has been followed in the case of the formation of the cistern; this (as we shall see) consists of the enlargement of the spaces of the periotic reticulum that originally occupied this region, the enlargement being a result of the disappearance of the protoplasmic bridges of the reticulum, whereby adjacent spaces unite in the formation of composite larger spaces. This process continues until there is a single continuous space extending down along the cochlear duct representing each *scala* and at the margins of each of them there is developed a membranous arrangement of the reticular cells which completely walls off the space from the surrounding tissue. In these alterations in the reticular mesh and in the formation of the surrounding membrane there is an active change in the form of the reticular cells, which repeatedly adapt themselves to the new conditions. There is no evidence to indicate that any other cells take part in the formation of the *scalæ*.

The first evidence of the formation of *scalæ* is found in fetuses about 40 mm. long, which stage is a little later than the first appearance of the cistern. In a fetus 43 mm. crown-rump length (Carnegie Collection, No. 886), along the proximal part of the cochlear duct on its basal surface there is a distinct widening of the meshes of the periotic reticulum. This is the beginning of the *scala tympani*. On the opposite side of the cochlear duct, where one would look for the *scala vestibuli*, the periotic reticulum retains its primitive appearance characterized by a narrow and rather uniform mesh. Thus the *scala tympani* makes its appearance slightly in advance of the *scala vestibuli*—that is, if we regard the latter as distinct from the cistern.

In fetuses 50 mm. long both the *scala tympani* and the *scala vestibuli* can be plainly identified, although they are still very incomplete. A wax-plate reconstruction of them, representing their form and their relation to the membranous labyrinth in a human fetus 50 mm. crown-rump length (Carnegie Collection, No. 84), is shown in figures 26 and 27, being a median and a lateral view respectively.

In preparing this and the models shown in figures 28 to 31, it is to be remembered that only those periotic spaces are included that were outlined by a membrane-like margin. In the adjacent reticulum there are spaces that are actively coalescing and gradually uniting with the main cavity. No attempt, however, was made to show such spaces in the models. From figures 26 and 27 it will be seen that the scala tympani is larger and more advanced in its development than the scala vestibuli. The latter is in its earliest stage and consists of hardly more than a row of enlarged reticular spaces which extend downward from the cistern along the dorsal and apical surface of the cochlear duct. A section through the scala vestibuli in another fetus of about the same age (Carnegie Collection, No. 448) is roughly shown in figure 21, the spaces of the scala being situated between the cistern and the cochlear duct.

The scala tympani consists of an elongated oval space lying along the basal surface of the proximal part of the cochlear duct, about corresponding to the proximal half of the first turn of the duct. In the main part it is a single space with a distinct margin separating it from the general periotic reticulum. In the more apical portion it tapers off into multiple incompletely united smaller spaces which actively coalesce as the process advances into the new territory along the duct. It is of interest to note that the most mature and the largest part of this scala, representing the focus at which it first appeared, is opposite the fenestra cochleæ (rotunda), just as the cistern forms opposite the stapes and the fenestra vestibuli. The scala tympani always begins at the same place and extends downward along the cochlear duct, at first a little in advance of the scala vestibuli, but subsequently the latter catches up with it and the two reach the tip of the duct at about the same time.

It is well known that the proximal portions of the cochlear duct mature sooner than the distal portions. One might expect that the accompanying periotic spaces would correspond in their development to the maturity of the duct and therefore the proximal parts of the scalæ would differentiate first. In other words, the maturation of the cochlea proceeds as a wave from the proximal end to its tip, involving all of its constituent structures as it passes along, including mesenchymal parts as well as epithelial.

This conception might explain the direction of development of the scalæ, but can hardly be applied to the cistern, the vestibular representative of the scala vestibuli. One can not say that those portions of the membranous labyrinth lying opposite the focus of development of the cistern (that is, the lateral walls of the saccule and utricle) mature in advance of the rest of the labyrinth. There is no indication that a wave of differentiation passes through the epithelial elements of the labyrinth in the same direction and synchronously with the extension of the cistern as it advances from its primary focus upon the roof of the utricle and over on its median surface. In the case of the cistern it seems much more likely that the point at which it first appears is determined by the position of the stapes, which is doubtless an expression of the physical relation that subsequently exists between the two. By analogy this would yield additional significance to the relation existing between the fenestra cochleæ and the point of beginning development of the scala tympani.

In dealing with the cistern and also with the *scalæ* one should not consider them as insignificant accessories that merely fill in the waste intervals between the membranous labyrinth and the surrounding cartilage. From studying their development it becomes apparent that they have a morphological individuality in many respects as definite as that of the ossicles themselves. They make their appearance at a definite time and at definite places, they spread in a definite manner, and eventually they attain a form and structure that are adapted to a definite function. This becomes more and more evident as we examine older stages.

The form and relations of the *scalæ* in fetuses between 12 and 13 weeks old are shown in figures 28 and 29. These figures show median and lateral views of a wax-plate reconstruction of the membranous labyrinth and the surrounding periotic spaces in a human fetus 85 mm. crown-rump length (Carnegie Collection, No. 1400-30). Attention has already been directed to these figures in the description previously given of the cistern. The *scala vestibuli* can be seen in figure 28. Above, it opens freely into the cistern and extends downward along the apical side of the duct as a single main space, possessing a rather uniform diameter. It extends along the first two turns of the duct, gradually tapering off and showing a less mature character in its distal portions. Along the second turn of the duct the spaces are incompletely fused and the contour becomes correspondingly irregular. As a rule the peripheral margin of the *scala* is less mature and more irregular than the central margin. The *scala vestibuli* does not connect with the *scala tympani* at any point as yet. The two are separated in the first place by the cochlear duct and then more centrally by a framework of connective tissue in which are the radiating bundles of the cochlear nerve with the nodes of ganglion cells that form the spinal ganglion. These latter structures are not shown in the model; they occupy, however, the V-shaped groove seen between the two *scalæ*.

The *scala tympani*, as can be seen in figure 29, extends downward on the basal side of the cochlear duct along its first two turns. This corresponds to about the same linear dimension as that of the *scala vestibuli*. In its proximal portion it shows a greater area in cross-section than the latter, but further toward the apical region it is of about the same size and in some places it is even smaller. The peripheral margin of the *scala tympani* is distinctly more irregular than the central margin. The irregularity is due to spaces along this margin that are actively coalescing with the main space, but in which the fusion is not yet complete. The irregularity of this margin is thus an indication of the direction of the expansion of the *scala*. As the diameter of the whole cochlear mass increases, it is evident that the main growth of the *scala* must radiate outward in a peripheral direction. This is accomplished by the continual assimilation of new reticular spaces along this margin. At the proximal end of the *scala tympani* can be seen an oval depression which corresponds to the *fenestra cochleæ* (*rotunda*) and with which it stands in intimate relation.

In fetuses about 16 weeks old the form and relations of the *scalæ* have nearly attained the adult conditions, and this represents the oldest stage studied in connection with the present paper. The conditions found at this time are shown

in figures 30 and 31, which present median and lateral views of a wax-plate model of a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018). On comparing the scalæ tympani and scalæ vestibuli as seen in these figures with those in figures 28 and 29, it will be seen that they are larger in cross-section and more nearly cover the cochlear duct. Furthermore, they now extend to the extreme tip of the duct and communicate with each other across its central margin, thus forming a helicotrema. A section through this point can be seen in figure 25, in which these structures are shown as seen under low magnification. It will be noted that now, even as far as the tip of the cochlea, each of the scalæ consists of a continuous principal space, though both are more mature and larger in their proximal portions. Along the first turn of the cochlear duct they are walled off by a smooth membranous margin which separates them from the adjacent reticular tissue. The spaces of the latter do not seem to be taking any further part in the process of enlargement of the scalæ. Along the second turn of the cochlear duct, a section of which is shown in figure 20, the coalescence of reticular spaces with each other and with the scalæ is still in active operation. This produces a greater irregularity of the scalæ than is shown in the model. The subsidiary spaces are shown as a solid mass; the slender clefts separating them are not represented. The nearer one approaches the tip of the duct the more immature are the scalæ, until the condition is reached that is shown in figure 25, where the membrane-like margin is quite incomplete and the spaces merge irregularly with the surrounding reticulum. Thus a single specimen, if studied in its different parts, shows several stages in this interesting process of the formation and growth of the scalæ.

The figures grouped on plate 3 illustrate some of the histological features of this process. An early stage in space-formation is shown in figure 23. This is a section through the canal region where the changes in the reticulum are late in making their appearance. In fact, the periotic spaces never reach the same degree of differentiation here that occurs in the case of the cistern and scalæ. The initial steps, however, are the same, and this figure presents very well the appearance of the periotic reticulum as it begins to open up into larger spaces. Unmodified reticulum is characterized by a rather uniform narrow mesh. The essential change in space-formation consists in the disappearance of some of the trabeculæ of the mesh, with the consequent coalescence of the corresponding adjacent spaces. The trabeculæ consist of the protoplasmic processes of the constituent cells of the reticulum and their disappearance is to be explained in either of two ways: It is possible that owing to some property of the fluid element of the tissue the protoplasmic strands are dissolved or liquefied; this would account for their complete disappearance. On the other hand, the same result could be accomplished by an alteration in the form of the cell processes. A given trabecula could separate at either end, or at some point along its line, and the free ends of protoplasm could then retract and reshape themselves and become a part of the remaining framework. Whether we are dealing with a liquefaction of tissue or with active motility of the cell, protoplasm involving detachment and retraction of the trabeculæ can

not be definitely determined by observations of fixed tissue; but the appearance of sections where the process is in active operation seems to the writer to indicate the latter.

In the above paragraph and elsewhere in this paper reference is made to trabeculæ serving as "partitions" between "spaces" and the disappearance of trabeculæ resulting in the "coalescence of adjacent spaces." In making this use of the term "space" it should be explained that it is done in a descriptive sense, in application to the appearance of the tissue as seen in sections in which form human embryological material is mainly available. In thin sections of a reticular tissue one sees trabeculæ as partitions separating adjacent spaces. The same tissue in a mass would show that the spaces everywhere communicate freely with each other, like the spaces in a sponge, and that the trabeculæ are thread-like strands which at the best are very incomplete partitions. Instead of a meshwork containing many small spaces, one could perhaps equally well describe reticular tissue as a single large space traversed by many trabeculæ. If the latter practice were adopted, one would describe the development of the tissue-spaces with which we are concerned as a process of gradual decrease in the number of traversing trabeculæ, with the result that the mesh thereby becomes coarser. For descriptive purposes, however, it is convenient to refer to the intervals between the strands of the mesh as spaces, at the same time not granting them the significance that is attached to such membrane-lined tissue-spaces as are represented by the vestibular cistern and the two scalæ, though the latter are in reality derived from them.

In figure 23 the free detached ends of the trabeculæ will be noted everywhere, as is characteristic of this stage of development. It is a necessary step in the coalescence of adjacent spaces. The detached trabeculæ seem to be gradually retracting and adapting themselves to the formation of larger spaces. Their constituent protoplasm reshapes itself as a smooth border or as a part of other trabeculæ. Larger spaces necessitate longer trabeculæ, and as trabeculæ become longer they also tend to become heavier. These phenomena are all in evidence in the spreading and enlargement of the scalæ.

Figure 20 shows a characteristic view of the scalæ as seen under low magnification. It will be noted that the scala vestibuli is relatively mature at this point; the scala tympani, however, is in the act of spreading peripherally, so as to underlie, as it eventually will do, the future basilar membrane. The scala tympani finally reaches the peripheral margin of the cochlear duct, and it does this by the coalescence of the enlarging reticular spaces, which become incorporated with the main cavity of the scala. This can be observed better in figure 22, which shows a detail of the same section as seen under higher magnification. By comparing this figure with figure 20 the exact location can be readily made out. A portion of the main cavity of the scala is indicated and to the right of this are a few enlarged reticular spaces that are uniting with each other and will in the end become part of the main space. In addition to the enlarged reticular spaces there is a certain amount of residual undifferentiated reticulum. It is this tissue that will play the

part of an adventitial coat to the completed scala. The trabeculae that separate the enlarged spaces seem to be under tension and about ready to snap apart. In fact, in most sections one can see the fragmentary ends of trabeculae where this interruption of continuity has apparently occurred.

The differentiation of the margin of the scala constitutes the final feature in their maturation. During the period in which the enlargement of an individual scala is being brought about by the coalescence of enlarging reticular spaces, the margins of the main cavity can be seen to consist of smooth, delicate strands of nucleated protoplasm that resembles in all essentials that of the trabeculae between the large reticular spaces. These linear margins are interrupted here and there by openings into adjacent spaces, but they tend to form a continuous line that definitely marks off the space from the adjacent reticulum. An early stage in the formation of such a margin is shown in figure 25, where the margin is indicated at a few places, but for the most part the space abuts against the surrounding ragged reticulum. The margin of the space is more complete in the scala tympani shown in figure 22, but it is still thin and delicate and can be easily opened up to allow the taking in of new spaces. If we examine the borders of more mature spaces we find them inclosed by a firmer membrane, which finally reaches a state that will probably not admit of any further opening up for the coalescence of additional spaces. Any further growth must thereafter be limited to simple distention of the wall of the space with the consequent adjustment of its constituent cells. Such a condition is represented in figure 24. This shows a more mature section of the wall of the scala vestibuli, being a detail of the same section shown in figure 20. The only difference between such a membrane, as we must now call it, and the corresponding structure in younger stages is its density; it is wider and its protoplasm perhaps more opaque, or in other words, more protoplasm is accumulated there.

If figures 24, 22, 25, and 23 are compared and followed in that order, it will be seen that the lining membrane of the scala can be traced backward, step by step, to the ordinary trabeculae of the periotic reticulum. There is no histological evidence that any new cells enter into its formation. It seems to be simply a product of the proliferation and adaptive reshaping of the cells already there. In its final form the margin of the space resembles an endothelial membrane. One could describe, as immediately lining the space, a thin membrane with flattened nuclei, which is supported underneath by a thin coat of nucleated protoplasm that has the form of fibrous connective tissue. The former, judging only from its final appearance, one might designate as endothelium and thus make a distinction between it and the underlying tissue. In its histogenesis, however, it differs in no way from the rest of the wall and the difference that exists later seems to be merely the result of its adaptation to the existing physical conditions. Its early behavior is entirely different from that of vascular endothelium. Thus if its final appearance is stressed and the term endothelium is used for its designation, it must be done with a considerable amount of reservation. It is preeminently a place where the term mesothelium could be used with great advantage.

COMMUNICATION OF PERIOTIC SPACES WITH ARACHNOID SPACES.

The relation of the scala tympani and scala vestibuli to the subarachnoid spaces surrounding the hind-brain is of considerable interest, both on account of the possibility of their functional relationship and on account of the similarity that exists in their development. For a satisfactory investigation of the establishment and the character of the communications that are formed between these two allied systems of tissue-spaces, one should resort to other methods than those used in the present study, and, furthermore, one should examine older fetuses than those described here. In fact, a problem lies here that would be well worth careful study.

Certain observations, however, were made in the course of the above investigation that bear relation to these matters, and they will be briefly outlined here. In the first place, the histological picture of the periotic reticulum is essentially the same as that of the early stages of the pia-arachnoidal tissue investing the central nervous system. The enlargement of the meshes of the latter and the formation of the subarachnoid spaces and the arachnoid cistern, as has been recently described by Weed (1917), correspond exactly with the appearance seen in the histogenesis of the periotic spaces in the ear. The periotic spaces are not, however, extensions of the arachnoid spaces that have invaded the cavity of the cartilaginous labyrinth. If this were so we should find them first appearing among the rootlets of the vestibular and cochlear nerves, along which the subarachnoid space extends for some little distance. Instead, they begin at points where there can be no connection with the arachnoid tissue and their direction of growth is quite independent of it. The periotic spaces may be analogous to the arachnoid spaces, but they are not identical with them, nor are they an extension of them.

According to the descriptions of the adult anatomy of the ear, a communication becomes established between the scala tympani and the subarachnoid space near the fenestra cochleæ, the so-called aquæductus cochleæ. Vague and conflicting statements are also made concerning a communication through the internal auditory meatus connecting the arachnoid spaces with the scalæ. Such communication must be established quite late. In the oldest fetus examined, 130 mm. crown-rump length, they did not yet exist. As to the latter communication, it can be seen that the arachnoid spaces extend peripherally through the internal auditory meatus along the trunk of the acoustic nerve-complex, and slender pockets and clefts from them extend along the larger bundles of the cochlear nerve; they terminate, however, before reaching the margins of the scalæ, and there is no evidence at this stage that there is ever to be a communication between them and the scalæ. As to the aquæductus cochleæ, in the 130 mm. fetus it can be plainly seen that it is already forming as a derivative of the arachnoid spaces, although the communication with the scala tympani is not yet established. The arachnoid spaces invest the glossopharyngeal nerve and extend down along its trunk and pass directly by the region of the fenestra cochleæ (rotunda). A thin-walled tubular pouch projects from these spaces, leaving the nerve trunk and extending obliquely toward the scala tympani in a direction that would meet it just distal to the fenestral

impression on its basal surface. This fundament of the aquæductus cochleæ is present in fetuses 85 mm. crown-rump length, but is longer in the 130 mm. fetus, where it nearly reaches the *scala tympani*. The communication must be established soon after this.

SUMMARY.

The changes in size and form which the cartilaginous capsule of the ear undergoes during its development in the human embryo are accomplished in part by a progressive and in part by a retrogressive differentiation of its constituent tissues. Throughout the entire period of growth, as far as material was available for study, it was found that the margins of the cartilaginous cavities undergo a process of continual transformation. They exhibit a state of unstable equilibrium in respect to the opposing tendencies toward a deposit of new cartilage on the one hand and toward the excavation of the old on the other. The margins thereby are always either advancing or receding, and it is in this way that the progressive alterations in the size, shape, and position of the cavities are produced, due to which a suitable suite of chambers is always provided for the enlarging membranous labyrinth.

The general tissue mass of the otic capsule, during the period represented by embryos from 4 to 30 mm. long, passes through three consecutive histogenetic periods, namely, the stage of mesenchymal syncytium, the stage of precartilage, and the stage of true cartilage. In the subsequent growth of the capsule it is found that in areas where new cartilage is being deposited the tissues of the areas concerned follow a definite and progressive order of development. In areas, however, where excavation occurs, where cartilage previously laid down is being removed, it is found that the process is reversed. The tissue in such areas returns to an earlier embryonic state—that is, it undergoes dedifferentiation. Tissue that has acquired all the histological characteristics of true cartilage can thus be traced in its reversion to precartilage and from precartilage in turn to a mesenchymal syncytium. In the latter form it redifferentiates into a more specialized tissue, in this case for the most part into a vascular reticulum.

The formation of the periotic reticulum is first indicated by a cluster of deeply staining nuclei that can be seen along the central edge of the semicircular ducts in embryos soon after the ducts are formed, and at about the time the otic capsule begins to change from condensed mesenchyme into precartilage. These nuclei constitute a focus at which the development of the reticulum and its blood-vessels takes origin. Here the tissue of the otic capsule takes on an appearance that is less like that of a cartilage-forming tissue and more like that of an embryonic connective tissue. Spreading from this focus, a narrow area is established which soon encircles the semicircular ducts and becomes the open-meshed vascular reticulum which, in embryos 30 mm. long, everywhere bridges the space existing between the epithelial labyrinth and the surrounding cartilage. In the earlier stages it could not be definitely shown that the primordium of the periotic reticular tissue is not derived from a few predestined mesenchyme cells which become inclosed, along with the otic vesicle, by the condensed tissue of the capsule and after a certain latent

period undergo proliferation and occupy the space vacated by the receding precartilagae. In the later stages, however, it is quite evident that precartilagae tissue is actually converted into a reticulum, and that the replacement of precartilagae by a reticular connective tissue is brought about through a process of dedifferentiation.

The perichondrium is a derivative of the periotic reticulum and forms an outer limiting membrane along its cartilaginous margin. During the fetal period the perichondrium does not rest directly against the true cartilage, but is separated from it by a zone of transitional tissue consisting partly of precartilagae and partly of reticulum. This transitional zone intervening between the perichondrium and the surrounding cartilage was observed in all of the specimens that were studied, which includes fetuses up to 130 mm. crown-rump length. Owing to the fact that the perichondrium is late in making its appearance, being first seen in fetuses about 70 mm. long, it can take no part in the early changes in the cartilaginous capsule, either as regards the deposit of new cartilage or the excavation of cartilage that had been previously laid down.

The periotic tissue-spaces are formed by a modification of the meshes of the periotic reticulum. The latter consists originally of a rather uniform narrow mesh. The essential change which it undergoes in the process of space-formation consists in the gradual disappearance of the traversing trabeculae. The trabeculae consist of the protoplasmic processes of the constituent cells of the reticulum, and their disappearance is apparently due, not to a dissolution or liquefaction of these cell-processes, but to an alteration in their form. It apparently is the result of an active motility of the cell protoplasm involving the successive detachment and retraction of the trabeculae. When a trabecula becomes detached it retracts and adapts itself to the formation of the enlarging space, reshaping itself either as a smooth border or as a constituent part of another trabecula.

The differentiation of the margin of the periotic spaces constitutes the final feature in their maturation. During the period in which the enlargement of an individual space is actively going on, the margins of the main cavity consist of smooth, delicate strands of nucleated protoplasm that resemble the trabeculae between the large reticular spaces. These linear margins are interrupted here and there by openings into adjacent spaces. They tend, however, to form a continuous line that definitely marks off the space from the adjacent reticulum. As the space becomes more mature, the membrane-like border becomes thicker until it reaches a state that will probably not admit of any further opening-up for the coalescence of additional spaces. Any further growth is thereafter limited to a simple distention of the wall of the space, with the consequent adjustment of its constituent cells. In its final form the margin of the space constitutes a mesothelial membrane. Immediately lining the space is a thin membrane with flattened nuclei which is supported underneath by a thin coat of nucleated protoplasm having the form of fibrous connective tissue. The former in its histogenesis differs in no way from the rest of the wall and the difference that exists later seems to be merely the result of its adaptation to the existing physical conditions.

The earliest histological evidence of the formation of the periotic spaces occurs near the stapes, in the reticulum that bridges the interval between the sacculus and the fenestra vestibuli. In embryos between 30 mm. and 40 mm. long, it can be seen that the meshes in this region are becoming irregular and larger, due to the disappearance of some of the trabeculae and a consequent coalescence of the intertrabecular spaces. The widening of the mesh at this point constitutes the primordium of the vestibular cistern. It makes its appearance before there is any trace of the scala, but it is not until the fetus reaches a length of about 50 mm. that the cistern becomes definitely outlined and clearly differentiated from the adjoining reticulum.

Following the appearance of the cistern, the scala tympani is the next space to become established. It can be recognized as a moderate widening of the meshes of the reticulum in the region of the fenestra cochleae in fetuses 43 mm. long, along the basal border of the first turn of the cochlear duct. The scala vestibuli, as can be seen in fetuses 50 mm. long, develops as an extension downward of the cistern along the apical border of the cochlear duct. Starting from these definite foci, these three spaces spread into their destined territory, absorbing as they go the enlarging reticular spaces of the invaded region by a process of space-coalescence, or, in other words, the progressive formation of areas that are free of trabeculae. In fetuses 85 mm. long the two scalae extend downward along the cochlear duct to its last turn, as two separate spaces which do not communicate with each other. When they reach the tip of the duct, which occurs in fetuses about 130 mm. crown-rump length, a free opening is developed between them which represents the helicotrema. After being completely established along the whole length of the cochlear duct, the scalae continue to enlarge by further coalescence of tissue along their peripheral border, in which the trabeculae disappear.

The periotic spaces are analogous in their development to the pia-arachnoidal spaces; they are not, however, extensions of them that have invaded the cavity of the cartilaginous labyrinth. They begin at points where there can be no connection with the arachnoidal tissue and their direction of growth is quite independent of it. The communication that is found in the adult between the scala tympani and the subarachnoid space in the neighborhood of the fenestra cochleae, the so-called aqueductus cochleae, is established quite late. In fetuses 85 mm. crown-rump length it exists as a tubular pouch projecting from the subarachnoid spaces along the glossopharyngeal nerve toward the scala tympani. In the 130-mm. fetus, the oldest examined, this pouch is longer and nearly reaches the scala. The communication must be established soon after this.

Similar projections from the subarachnoid spaces at the internal auditory meatus extend as perineural clefts along the trunk and branches of the acoustic nerve. No actual communications, however, were seen between these spaces and the two scalae.

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EXPLANATION OF PLATES.

PLATE 1.

- The figures on Plates I and II represent a series of photographs of the ear region in human embryos varying from 4 mm. to 130 mm. long. The photographs were taken at a magnification of 100 diameters and as far as possible at similar positions, so that a comparison of them would indicate the actual increase in size and the relative amount and form of the individual tissue-masses. In the reproduction they were reduced to about 90 diameters. The different figures include the principal stages in the development of the cartilaginous capsule of the ear and show the gross features of the histogenesis of the periotic reticulum. Figures 5 to 7 cover the period during which the mesenchyme becomes condensed around the otic vesicle. Figures 8 to 10 show the otic capsule in its precartilag stage and the manner in which the precartilag becomes differentiated into relatively permanent and temporary zones. The latter encircle the epithelial ducts and correspond to the future cartilaginous canals. In figures 11 to 13 the main capsular mass has become true cartilage, whereas the temporary zone of precartilag surrounding the canal is on the point of dedifferentiating into periotic reticulum. A focal area of vascularized reticulum is already established at the inner margin of the epithelial duct.
- FIG. 5. Frontal section through the region of the ear in a human embryo 4 mm. long (Carnegie Collection, No. 588, slide 6, row 6, section 6). The section is 15μ thick and is enlarged 90 diameters. It shows part of the brain-wall and the otic vesicle with the surrounding mesenchyme. The nuclei of the latter are more numerous in the neighborhood of the vesicle, indicating the beginning of the capsular condensation.
- FIG. 6. Horizontal section through the region of the ear in a human embryo 9 mm. long (Carnegie Collection, No. 721, slide 5, row 2, section 1). The section is 15μ thick and is enlarged 90 diameters. It shows a distinct condensation of the mesenchyme around the otic vesicle, particularly on its lateral surface (above) where it extends from the surface of the vesicle to about half the distance from the vesicle to the ectoderm.
- FIG. 7. Frontal section through the labyrinth in a human embryo 11 mm. long (Carnegie Collection, No. 353, slide 16, row 3, section 4). The section is 10μ thick and is enlarged 90 diameters. It shows the vestibular part of the labyrinth with the appendage opening out of it and passes transversely through the pouches whose margins are to form the superior and lateral semicircular ducts. There is now a very complete capsule of condensed mesenchyme surrounding every part of the labyrinth, with the exception of the appendage and the regions of the internal auditory meatus and the fenestra cochleæ.
- FIG. 8. Horizontal section through the otic capsule in a human embryo 15 mm. long (Carnegie Collection, No. 719, slide 3, row 2, section 3). The section is 40μ thick and is enlarged 90 diameters. It shows a portion of the utricle below and the superior semicircular duct above. Surrounding these is a definite capsule of precartilag tissue.
- FIG. 9. Sagittal section through the otic capsule in a human embryo 18 mm. long (Carnegie Collection, No. 144, slide 4, row 1, section 3). The section is 40μ thick and is enlarged 90 diameters. Above is the posterior semicircular duct, and just below the center is the lateral semicircular duct. The otic capsule is now differentiated into relatively permanent areas of precartilag and other areas that are more temporary. The latter surround the epithelial ducts and indicate the future cartilaginous canals.
- FIG. 10. Frontal section through the otic capsule in a human embryo 27 mm. crown-rump length (Carnegie Collection, No. 756a, slide 47, section 2). The section is 50μ thick and is enlarged 90 diameters. It passes transversely through the lateral semicircular canal. The epithelial duct is surrounded by a zone of temporary precartilag corresponding to the future cartilaginous canal. Just median to the duct (below it in the photograph) is a group of nuclei that forms the focus of the future growth of reticulum.
- FIG. 11. Section through the lateral semicircular canal in a human fetus 30 mm. crown-rump (Carnegie Collection, No. 86, slide 46, section 2). The section is 50μ thick and is enlarged 90 diameters. The main capsular mass is now differentiated into true cartilage. The zone of temporary precartilag is beginning to recede from the epithelial duct, leaving a reticular area in the interval, which is more pronounced on the median side of the duct (below it in the photograph).
- FIG. 12. Section through the lateral semicircular canal in a human fetus 37 mm. crown-rump length (Carnegie Collection, No. 972, slide 20, section 1). The section is 50μ thick and is enlarged 90 diameters. The nuclei of the zone of temporary precartilag form a dark field that corresponds to the future cartilaginous canal. Along the inner margin of this zone are seen large blood-vessels that belong to the periotic reticulum.
- FIG. 13. Section through the lateral semicircular canal in a human fetus 35 mm. crown-rump length (Carnegie Collection, No. 199, slide 58, section 2). The section is 50μ thick and is enlarged 90 diameters. It is stained deeply with hematoxylin, showing the matrix of the cartilage but not the zone of precartilag that is to become the cartilaginous canal.

PLATE 2.

The figures on Plate II are in continuation of those on Plate I and show the final establishment of the periotic reticular tissue. They also show, on being compared with younger stages, the manner in which the cartilage becomes excavated in order to yield room for the enlarging duct and also to allow for its changing position. The excavation is brought about by the dedifferentiation of cartilage into reticular tissue. Throughout this period the margin of the cartilaginous canal continues in an unstable condition and is gradually either

receding or advancing, through the processes of dedifferentiation, into precartilage or differentiation from precartilage respectively. The periotic reticulum in its later stages develops fibrous membranes at its inner and outer borders. The one at the inner border forms the membrana propria for the epithelial duct, and the one at the outer border becomes the perichondrium.

- Fig. 14. Section through the lateral semicircular canal in a human fetus 43 mm. crown-rump length (Carnegie Collection, No. 886, slide 42, section 3). The section is 100μ thick and is enlarged 90 diameters. The zone of precartilage is expanding around its peripheral margin by dedifferentiation of the surrounding cartilage and on its central margin the precartilage is giving way before the advancing reticulum. A crescentic area of periotic reticulum is established on the median side (to the left) of the epithelial duct, about 8 mm. deep in the photograph.
- Fig. 15. Section through the lateral semicircular canal in a human fetus 46 mm. crown-rump length (Carnegie Collection, No. 95, slide 72, section 1). The section is 100μ thick and is enlarged 90 diameters. The original area of precartilage is now all dedifferentiated into reticulum, and a new area of precartilage has formed outside of this at the expense of the surrounding cartilage. The new area of precartilage is about 0.8 cm. deep in the photograph. Everything between this and the epithelium is reticulum, the peripheral part of which is not yet completely vascularized.
- Fig. 16. Section through the posterior semicircular canal in a human fetus 50 mm. crown-rump length (Carnegie Collection, No. 184, slide 23). The section is 50μ thick and is enlarged 90 diameters. The dedifferentiation of precartilage into reticulum is nearly complete, there being left only a narrow line of it along the margin of the cartilage. The vascularization of the reticulum is not yet completed. The small diameter and the thick wall of the epithelial duct in this figure and in figure 15 result from contraction. If they were distended in the process of fixation they would doubtless be as large as those in figures 14 and 17.
- Fig. 17. Section through the posterior semicircular canal in a human fetus 52 mm. crown-rump length (Carnegie Collection, No. 96, slide 12, section 2). The section is 100μ thick and is enlarged 90 diameters. It differs from figure 16 in having a more mature periotic reticulum.
- Fig. 18. Section through the posterior semicircular canal in a human fetus, 85 mm. crown-rump length (Carnegie Collection, No. 1400-30, slide 43, section 2). The section is 100μ thick and is enlarged 90 diameters. At the inner margin of the reticulum can now be seen the membrana propria supporting the semicircular duct and at the outer margin is the thick peri-hondrium, between which and the cartilage there is a narrow open space that is better seen on the left part of the photograph. The sharp dark line along the margin of the cartilage on the right is an appearance due to the excavation of cartilage at that point. It consists of an intermediate zone in which the cartilage is being dedifferentiated into precartilage and that in turn into reticular tissue.
- Fig. 19. Section through the superior semicircular canal in a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018, slide 30, section 1). The section is 50μ thick and is enlarged 90 diameters. It shows a rather mature perichondrium closely attached to the cartilage, separated from it, however, by a narrow intermediate zone that is not seen in the photograph. This zone is connected with the further enlargement of the cartilaginous canal, the growth of which is not yet completed. In the outer part of the canal the perichondrium fuses with the membrana propria of the semicircular duct. The periotic reticulum is beginning to break up in the formation of larger spaces, which it does by the retraction of its trabeculae, thereby allowing adjacent spaces to coalesce. The blood-vessels in this specimen were injected with India ink.

PLATE 3.

The figures on Plate III show the histological appearance of the periotic tissue-spaces and the manner in which they are formed from the periotic reticulum. This is accomplished by the disappearance of the trabeculae and the consequent repeated coalescence of adjoining spaces.

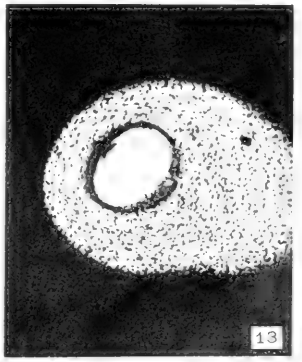
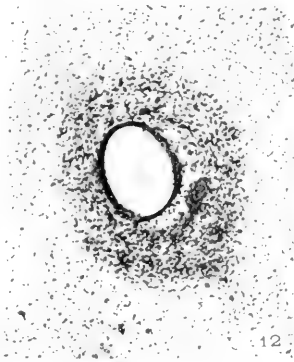
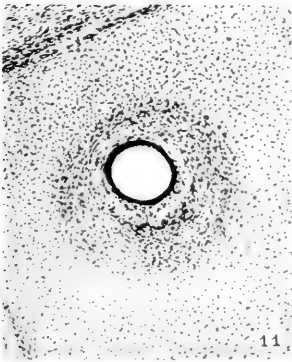
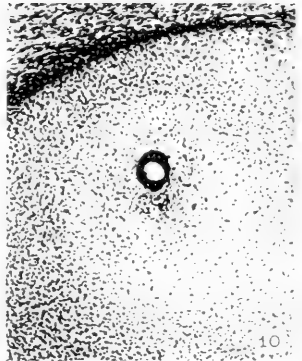
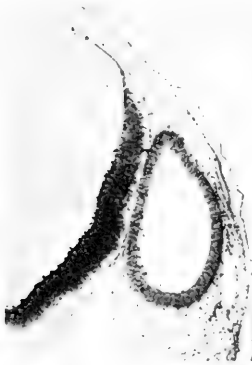
- Fig. 20. Section through the second turn of the cochlea in a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018, slide 32, section 2), enlarged 57 diameters. This section shows the topography of the cochlear duct and the general character of the periotic spaces that are developing along its inner margins. Details of this same section as seen under higher magnification are shown in figures 22 and 24.
- Fig. 22. Detail of the section shown in figure 20, enlarged 278 diameters. This figure shows the part of the cochlear duct that is to form the organ of Corti with the adjacent tissue that becomes incorporated in the basilar membrane. Below is the periotic reticulum, whose spaces are in the process of enlarging. By repeated coalescence these spaces finally unite with the large space which constitutes the scala tympani. This figure shows the histological appearance of the reticulum where the formation of tissue-spaces is in active operation.
- Fig. 24. Detail of the section shown in figure 20, enlarged 300 diameters. It shows the character of the margin of the scala vestibuli in a fairly mature condition. The scala vestibuli is inclosed by a membrane consisting of the cells that had previously constituted the reticulum occupying this area and which have been modified in form in adaptation to the formation of this large tissue-space, closing it off from the surrounding tissue.
- Fig. 21. Section through the vestibular portion of the labyrinth in a human fetus 52 mm. crown-rump length (Carnegie Collection, No. 448, slide 154, section 2), enlarged 31 diameters. This section shows the general character of the periotic spaces and their relation to the different parts of the membranous labyrinth and the surrounding cartilaginous capsule. The first space to develop and the largest shown in this figure is the vestibular cistern, situated between the utricle and the cartilaginous stapes. The smaller spaces, below the cistern and extending downward along the cochlear duct, represent the scala vestibuli in an early form. The arteries in this specimen were injected with India ink and are shown in black.

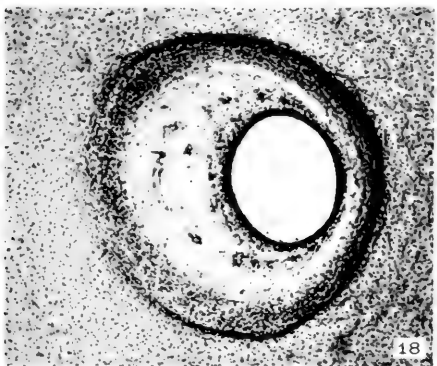
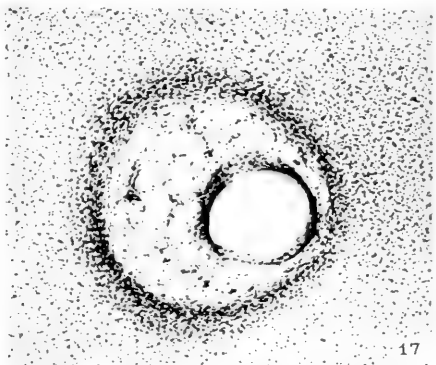
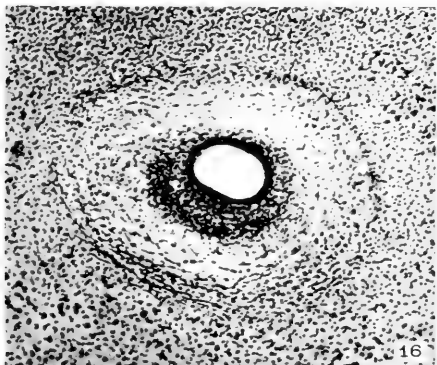
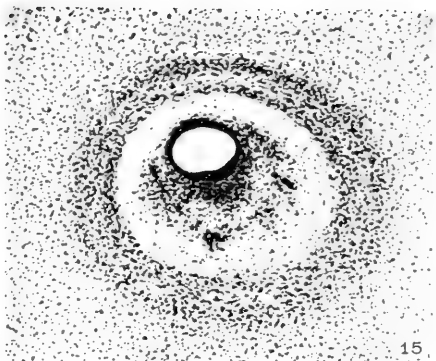
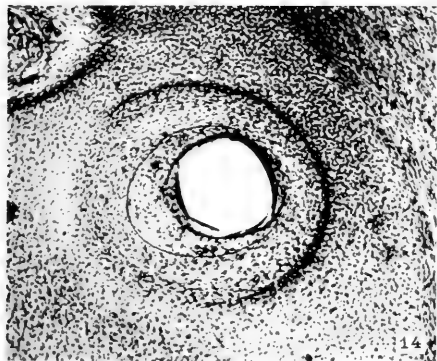
- FIG. 23. Section through the superior semicircular canal in a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018, slide 29, section 2), enlarged 90 diameters. The periotic reticulum is undergoing the alterations characteristic of the early stages of the formation of tissue-spaces. Along the margins of the cartilage the reticular tissue is condensed and constitutes the fibrous perichondrium. Around the epithelial canal there is developed a layer of supporting tissue which forms the membrana propria. This layer fuses with the perichondrium along the peripheral margin of the canal and thereby constitutes a ligament that attaches each membranous duct throughout its whole length to the cartilaginous space in which it is suspended.
- FIG. 25. Section through the apex of the cochlea of a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018, slide 32, section 2), enlarged 57 diameters. This section shows the tip of the cochlear duct and the character of the communication that develops between the two scala forming the helicotrema. It will be seen that the margins of the periotic spaces are not so mature here as in the proximal parts of the cochlea of the same fetus, on comparing this figure with figure 20.

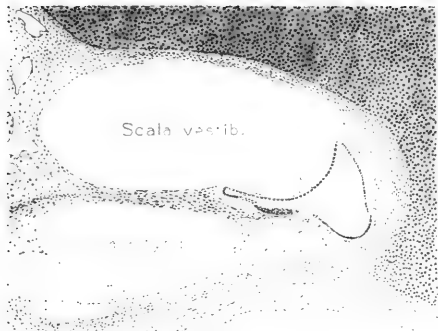
PLATE 4.

The figures shown on this plate represent a series of median and lateral views of wax-plate reconstructions of the membranous labyrinth and the surrounding periotic tissue-spaces. They illustrate under the same scale of enlargement three typical stages in the development of these spaces. Abbreviations: C. s. l., ductus semicircularis lateralis; C. s. p., ductus semicircularis posterior; C. s. s., ductus semicircularis superior; Duct. coch., ductus cochlearis; Impressio rotund., area opposite the fenestra cochleæ; Impressio staped., area in contact with base of stapes; Saccus endol., saccus endolymphaticus; Scala tympani, scala tympani; Scala vestibuli, scala vestibuli.

- FIG. 26. Lateral view of a model reconstructed from a human fetus 50 mm. crown-rump length (Carnegie Collection, No. 84). The cistern and the scala vestibuli are shown in green and the scala tympani is shown in orange. The scala vestibuli is in the first stage of its development and consists of a row of large reticular spaces which extend from the ventral margin of the cistern downward along the apical surface of the cochlear duct. The scala tympani is more advanced and shows more complete coalescence of its constituent spaces. Enlarged 11.4 diameters.
- FIG. 27. Median view of the same model shown in figure 26. This view shows the topography of the scala tympani. Its large proximal end lies opposite the fenestra cochleæ (rotunda) and corresponds to the focus at which its development originates. Distally it tapers off rapidly where the spaces are smaller and their coalescence less complete. Enlarged 11.4 diameters.
- FIG. 28. Lateral view of wax-plate reconstruction of the left membranous labyrinth and the periotic spaces in a human fetus 85 mm. crown-rump length (Carnegie Collection, No. 1400-30), enlarged 11.4 diameters. The cistern and the connecting scala vestibuli are shown in green. Although the greater part of the cistern abuts against the stapes, it will be noted that it is also beginning to spread over the dorsal surface of the utricle and along the inner border of the lateral semicircular duct. The scala vestibuli communicates freely with the cistern and extends downward along the apical surface of the cochlear duct throughout nearly two turns, showing the characteristic sacculated appearance near its tip, where the coalescence of the spaces is less complete.
- FIG. 29. Median view of same model shown in figure 28, enlarged 11.4 diameters. The scala tympani is shown in orange. The oval indentation in its proximal end corresponds to the fenestra cochleæ (rotunda). This space extends along the cochlear duct about the same distance as the scala vestibuli, but the two do not communicate yet at any place. The peripheral border of the scala tympani is characterized by sacculations corresponding to spaces that are coalescing with the main space. The growth of the scala is due to a coalescence of new spaces along its peripheral border rather than along its central border.
- FIG. 30. Lateral view of a wax-plate reconstruction of the left membranous labyrinth and the periotic spaces in a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018), enlarged 11.4 diameters. The cistern and scala vestibuli are shown in green and the scala tympani is shown in orange, as in the previous figures. The cartilaginous stapes was removed from this model and the oval impression that it makes on the cistern can be plainly seen. The cistern has spread over the top of the utricle and part way along the lateral semicircular duct. The scala vestibuli extends to the tip of the cochlear duct, where it communicates with the scala tympani, thus forming the helicotrema.
- FIG. 31. Median view of same model shown in figure 30, enlarged 11.4 diameters. The oval impression on the proximal end of the scala tympani corresponds to the fenestra cochleæ (rotunda). As yet there is no communication at this point between the scala tympani and subarachnoid spaces, such as is found in the adult and known as the aqueductus cochleæ. The spaces making up the cistern cover almost the whole of the utricle and saccus except the places at which the nerves enter and a small part of the medial surface near the attachment of the appendage.



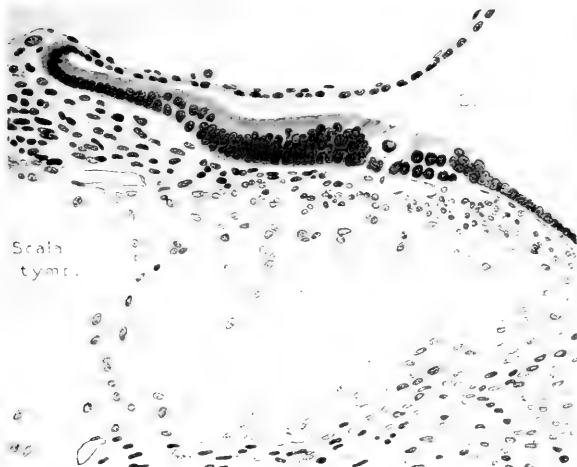




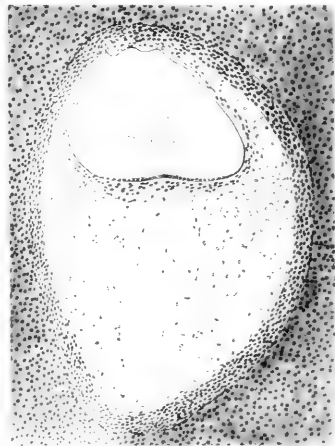
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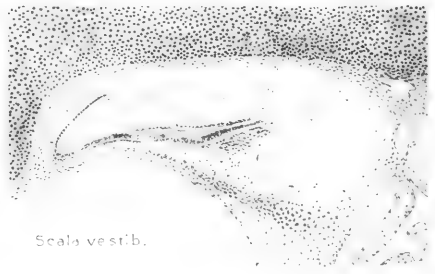
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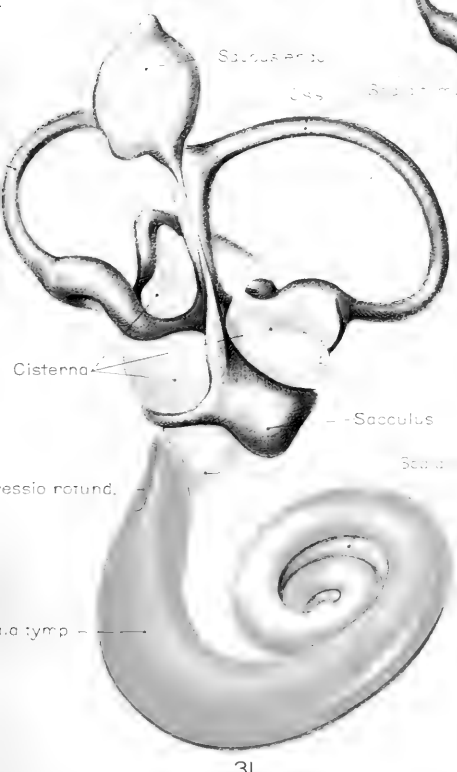
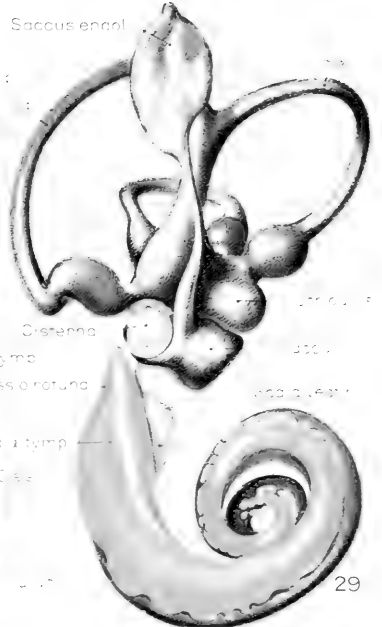
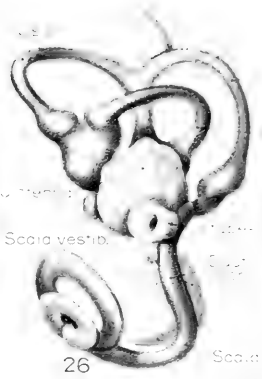
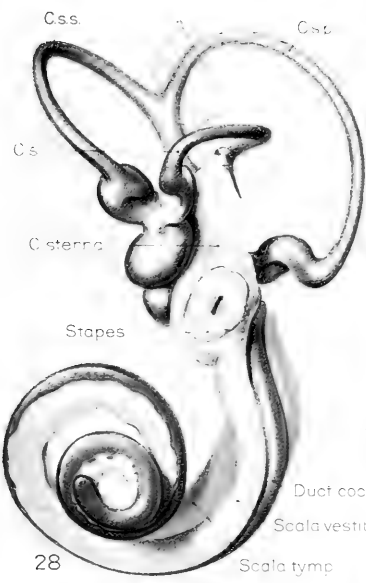
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CONTRIBUTIONS TO EMBRYOLOGY, No. 21.

THE GENESIS AND STRUCTURE OF THE MEMBRANA TECTORIA
AND THE CRISTA SPIRALIS OF THE COCHLEA.

BY O. VAN DER STRICHT.

With four plates (or thirty-six figures).

THE GENESIS AND STRUCTURE OF THE MEMBRANA TECTORIA AND THE CRISTA SPIRALIS OF THE COCHLEA.

BY O. VAN DER STRICHT.

INTRODUCTION.

The membrana tectoria belongs to a group of organs produced at the surface of the epithelium and termed superficial cuticles or superficial cuticular formations. Once developed, the generating epithelium persists in its entirety beneath the cuticle or exceptionally may disappear, as in the case of the ameloblasts, which atrophy after forming the enamel at their bases.

One may subdivide these structures into three groups: In the first the process of development can not be doubted or denied. It occurs just within the superficial layer of the cytoplasm and the cuticle produced remains in close contact, even continuity, with the generating cells. Examples are the striated borders of the columnar epithelium of the intestine, of the crypts of Lieberkühn, of the convoluted tubules of the kidney, of the syncytial layer of chorionic villi in the human placenta, and of osteoclasts.

The second is represented by the series of reticulares or fenestrated membranes covering the surface of sensorial epithelia—for example, the reticular membrane of the crista—and macula acustica and the organ of Corti, the membrana limitans externa of the retina, the membrana limitans olfactoria. The openings of the membrane are traversed by the apices of sensorial cells, the hairs of the acoustic cells, the rods and cones of the retina, the ciliated vesicles of the olfactory cells. These membranes in adult sensorial organs are in close contact with the surface of the epithelium, but are completely separated from the generating substratum. Hence their origin must be studied during the embryonic period of their development. Many authors regard them as a real cuticle derived from the free surfaces of the subjacent, that is to say, sustentacular cells. N. Van der Stricht (1908) has demonstrated that the reticular membrane of the acoustic epithelium is formed by a system of terminal bars closing the intercellular spaces between the embryonic epithelial cells. G. Leboucq (1909) proved that the membrana limitans externa of the retina is not formed at all by the Müller cells, but by the closing bars separating the apices of the rods and cones and Müller cells. The present writer (1909) found the membrana limitans olfactoria to have a similar origin. The zona pellucida surrounding the ovarian ovum in mammals and traversed by the prolongations of follicular cells which reach the surface of the egg must be considered as a fenestrated membrane of the same nature. According to the investigations of Dubreuil and Regaud (1908), it is derived from exoplasmic fibers produced within the intercellular spaces of the follicular cells. My own preparations of ovaries of

bats and dogs show that it is formed by the terminal bars, and Alice Thing (1917) considers that the very thick zona pellucida of the ovum of the turtle is produced by the terminal bars of the surrounding epithelium. They extend over the free surfaces of these cells, where appears a delicate network of the same nature as that of the bars. This network, together with the bars, gives rise to the cuticular fundamental substance of the zona pellucida.

Enamel and the membrana Corti or membrana tectoria are included in a third group. In both cases the adult organ becomes completely detached from its generating substratum, the first from the bases of the ameloblasts, each of which produces a kind of cuticular prism (the enamel prism). These elements are separated by the calcified cement substance which is considered to be a kind of intercellular product, although its origin has not been clearly described. The second, the membrana tectoria, becomes detached from the surface of the greater and the lesser epithelial ridges in the cochlear duct and remains fixed to only the least active portion of its generating substratum, the crista spiralis. Held (1909), discussing the nature of the membrana Corti, thinks that the membrane should not be considered cuticular, not because its layer first formed is not homogeneous, but because its constituent elements, its fibrils, as they become more and more elongated, proceed from the cytoplasm as different plasmic products and not as cell prolongations. A cuticle, he states, is not represented by flagella, by cilia of a ciliated epithelium, or by sensorial hairs. In addition a cuticle always remains attached to the surface of the cell. Hence Held regards the membrana tectoria as a specific product of the free surface of the greater and lesser epithelial ridges, the sensorial cells of which do not take part in its development. Therefore the fibrils of the membrana Corti can not be termed cuticular. Held seems to forget the recognized fact that enamel prisms are real cuticular elements, although they become completely detached from their anatomical substratum.

The object of my research is the study of the development and structure of the membrana tectoria. Although this problem has received the attention of many investigators, it seems to me that the results obtained have been rather contradictory and give for the most part no satisfactory interpretation because of differences between the morphological substratum and the real structure of the membrane derived from it. Recent investigators have more or less neglected the structure of the crista spiralis. I intend to devote to it special attention.

METHODS.

I have investigated the following material: Pig embryos of 60.0, 93.5, 95.0, 127.0, 137.0, 150.0, and 190.0 mm.; a new-born dog; young kittens; the following adult animals: bat, dog, rat, and mouse.

The isolated cochlea was fixed by one of the following agents: Trichloroacetic acid, 5 per cent in water; this decalcifies bone very well after one, two, or three days, according to the size of the cochlea; Bouin's fluid; Zenker's fluid.

After fixation by the first agent, the pieces were transmitted directly to absolute alcohol, to which some drops of iodine had been added. After remaining one

or two days in the second or third fluid, the pieces were washed in running water and hardened for many weeks in 70 per cent alcohol with some drops of iodine. The iodine effectively acts as a mordant. Where necessary, decalcification was completed by 2 per cent nitric acid in 70 per cent alcohol.

Before embedding in paraffin the pieces were stained by borax carmine. The series was then stained by iron hematoxylin, Congo red, and light green. In advanced stages of development the best results were usually obtained by the following treatment:

1. Immerse one day in 2.5 gm. ferric alum in 100 c.c. distilled water.
2. Wash one second in distilled water.
3. Immerse for some minutes in a solution of Congo red, 1 gm. in distilled water 200 c.c.
4. Wash in distilled water.
5. Immerse one day in a 0.5 per cent aqueous solution of crystallized hematoxylin.
6. Decolorize by a 1 per cent aqueous solution of ferric alum.
7. Wash for one hour in running water.
8. Stain for some seconds in a solution of 0.5 gm. light green in 200 c.c. of 95 per cent alcohol.
9. Treat in succession with absolute alcohol, xylol, and Canada balsam.

By this method the nuclei, the central corpuseles, and the terminal bars are stained very dark blue, the cytoplasm and its prolongations red, the ground substance of the connective tissue and the membrana tectoria green.

Mallory's method is also very useful for staining blue the ground substance of the connective tissue and the membrane of Corti.

The membrana tectoria possesses very delicate structures in which shrinkage and agglutination are provoked by the best fixing agents, although some of my series give results which are good and are confirmed by a new and better method tried during the past few weeks. Before treatment by one of the three fixing fluids mentioned above, I made one or two small openings in the bony wall of the cochlea and exposed the piece for 15 minutes to vapors from an aqueous solution of osmic acid or submerged it in a 1 per cent aqueous solution of the same for one hour. Afterwards fixation was completed by immersion in trichloroacetic acid, Bouin's fluid, or Zenker's fluid and the series of sections was stained by iron hematoxylin, Congo red, and light green. By this method some of the turns of the cochlea give very good preparations of the structure of the membrana tectoria. The mitochondria also are visible within osteoblasts, osteoclasts, connective-tissue cells, all epithelial cells, and the sensorial elements.

ANATOMICAL SUBSTRATUM OF THE MEMBRANA TECTORIA.

In spite of numerous investigations many features remain obscure in the histogenesis and structure of the membrana tectoria, in its connections with the adult organ of Corti, and in its extension beyond the sensorial epithelium. Embryologists almost all agree that the membrana tectoria appears in the earliest stages of development of the membranous cochlea before the appearance of the greater and the lesser epithelial ridges and the crista spiralis, as a kind of very thin membrane

on the surface of the somewhat thick epithelial layer covering the interior wall of the ductus cochlearis next to the scala tympani. While the two epithelial ridges and the crista spiralis are developing, the membrane thickens and is in close contact with the surface of their superficial epithelium, which I consider as the generating substratum, the matrix of the membrane of Corti, according to the investigations of most authors (Boettcher 1869, Nuel 1878, Pritchard 1876, Retzius 1884, Denis 1901, Rickenbacher 1901, Held 1909). Others believe that the process of formation extends farther over the surface of Hensen cells and Claudius cells (Hensen 1871, Tafani 1882, Dupuis 1894, Coyne and Cannieu 1895, Czinner and Hammerslag 1898, Vasticar 1909, Prentiss 1913). Previous authors asserted that the membrana tectoria reaches and is attached to the ligamentum spirale (Corti 1851, Claudius 1855, Boettcher 1859, Henle 1866, Loewenberg 1868, Barth 1889). Everyone who has studied this question has recognized that the greater ridge is the most active segment of this substratum; the crista spiralis, at the surface of which the membrane remains fixed in the adult cochlea, is of less significance; indeed, its activity ceases before the stage of complete development of the organ of Corti. A few authors, with Koelliker (1859), assert that the lesser ridge does not take part in the formation of the membrane, and Vernieuwe (1905) and Hardesty (1908, 1915) attribute very little importance to it.

Most investigators concur in regarding the membrana tectoria as a cuticular product of the cytoplasmic apices of the surface epithelial cells (Koelliker 1861, Hensen 1863, Middendorp 1867, Rosenberg 1868, Winiwarter 1870, Gottstein 1870, Pritchard 1876, Nuel 1878, Retzius 1884, Coyne and Cannieu 1895, Denis 1901, Rickenbacher 1901, Vernieuwe 1905, Hardesty 1908, Vasticar 1909, Prentiss 1913); whereas some (Boettcher 1869, Ayers 1891, Czinner and Hammerslag 1898) consider it to be produced by hairs, cilia, or filaments. Held (1909) practically confirms this opinion. I must, therefore, consider the apices of the superficial epithelium which enter into this process of genesis.

THE GREATER AND LESSER EPITHELIAL RIDGES.

On a transverse section of the tympanic wall of the cochlear duct, in the earliest stages before the appearance of the crista spiralis (pig embryo 60 mm.), and later, when the crista spiralis and the two ridges are visible but before any trace of differentiation has taken place in the sensorial elements, the wall of these regions is lined by a rather thick epithelium which was regarded by certain earlier authors (Koelliker 1859, Middendorp 1867, etc.) as formed by superposition of many rows of cells. But Hensen (1863), Boettcher (1869), Baginsky (1886), and other more recent investigators describe it as a simple columnar epithelium. The elongated prismatic cells reach the inferior and superficial part of the layer and their nuclei are situated at various heights. Figure 1, from a new-born dog, shows such a section near the top of the cochlea, with the first indication of the greater ridge (*gr*) and with the future lesser ridge (*lr*) not yet prominent. A superficial mosaic is visible at *mg*, *ml*, on the two segments, but without any differentiation in the sensorial fields. Many rows of nuclei (*n*) are apparent in the segment of the future lesser epithelial ridge.

In figure 2 are displayed the same details in a transverse, slightly oblique section of the second turn of the cochlea in a pig embryo (93.5 mm.). The lesser epithelial ridge is barely indicated (*lr*) by a superficial mosaic (*ml*), of which all the polygons belong to indifferent epithelial cells, although below them are seen three special nuclei (*ns*) separated by a considerable distance from others more deeply situated (*nsu*). This superficial location of the nucleus is the first sign of sensorial differentiation in an epithelial cell. The deep nuclei belong to future supporting cells. The superficial mosaic of the greater ridge is visible (*mg*), and just at its lateral or outer side is a row of five fields, three larger (*ih*) separated by two smaller (*is*), three apices of future inner hair-cells separated by two apices of future inner supporting cells. The outer hair-cells appear later. Toward the axial part of the greater ridge exist three mitotic figures (*mi*) located below the superficial mosaic; they are the last existing traces of the proliferation zone of Baginsky (1886), which is very well marked in earlier stages.

Figure 3 represents a transverse, slightly oblique section of the greater (*gr*) and lesser (*lr*) ridges on the second turn of the cochlea in a new-born dog. N. Van der Stricht (1908) describes many similar figures in his photos 42, 42', 44, 45. I will emphasize only the details reproduced on a greater scale in figure 4, a section tangential to the surface of the two epithelial ridges. These confirm for the dog the description given by N. Van der Stricht of embryonic bats, as shown in his photo 52 among others.

From the axial towards the outer region of the two ridges in figure 4 are the following:

1. The superficial mosaic of the greater ridge (*mg*), the most lateral polygons of which are differentiated into one row of circular inner sensorial fields, the apices of the inner hair-cells (*ih*) regularly separated by compressed elongated narrow fields, the apices of the inner supporting cells (*is*).
2. A row of small polygons, the apices of the inner pillars (*ip*).
3. A first row of apices of outer hair-cells (*oh'*) separated by the phalanges of the outer pillars (*op*).
4. A second row of apices of outer hair-cells (*oh''*) separated by the phalanges of the first row of Deiters cells (*d'*).
5. A third row of apices of outer hair-cells (*oh'''*) separated by the phalanges of the second row of Deiters cells (*d''*).
6. The apices of the third row of Deiters cells in the form of small polygons (*d'''*) similar to those of the inner pillars.

Figure 5 is from a segment between the second and third turns of the same cochlea and shows identical structures. But there exists a fourth row of outer hair-cells (*oh^{iv}*) and a fourth row of Deiters cells (*d^{iv}*) along a very small portion of the cochlea. Retzius (1884) states that the organ of Corti in the rabbit and the dog exhibits a fourth row of outer hair-cells in the superior part of the middle turn and along the largest part of the apical turn. Waldeyer (1872) mentions a fourth row in man and Retzius (1884) confirms this, but adds that the fourth row and even a fifth belong to the upper part of the cochlea and are largely interrupted. I must point out that the outer sensorial fields of the recently differentiated part

of the organ of Corti are much smaller than the older inner fields (*ih*, fig. 4). In figure 5 all the apices of the hair-cells are of the same size and the superficial horseshoes (*hs*), cut from their subjacent cuticular dark plate from which the hairs proceed, are more clearly visible.

If figures 1, 2, 3, 4, and 5 be carefully compared with one another and with other similar epithelial areas in the cochlea duct, it will be observed that the polygons vary in size. In the first stages of development (figs. 1 and 2) they are largest on the surface of the greater epithelial ridge and much smaller on the future lesser thickening, where their size does not exceed that of the apices of the Hensen cells (*mh*). But in more advanced stages the fields of the crista spiralis (*mer*, fig. 6) and of the Hensen cells become the largest. They do not alter very much on the surface of the greater thickening, although when the organ of Corti is differentiated they extend a little and retain this size more or less until the membrana tectoria becomes detached from its anatomical substratum (fig. 6, *ssp*). But now they (*mg*) manifestly decrease along an inner segment (*min*) of the greater ridge near the vestibular lip (fig. 6). Figure 24 (N. Van der Stricht) shows this detail much better than my figure 6.

Figure 4 shows the alterations already mentioned as undergone by the primitive small polygons (*ml*) in figures 1 and 2 when the sensorial (fig. 4) and supporting fields appear. During the development of the membrana tectoria they remain more or less unchanged, as may be seen in figure 5; but before reaching the stages of the adult organ of Corti, gradual transformations occur at the apices of the inner and outer pillars and in the terminal bars which form the superficial membrana reticulatis, as described by N. Van der Stricht. To recall details discussed by this author I will give figure 7, which shows the apices of all the constituents of an adult organ of Corti in the bat, in order to demonstrate in connection with the fields of the inner supporting cells a fact of some importance, upon which I shall dwell later, when I speak of the terminal bars.

Within each area of the indifferent mosaic of the greater ridge exists a central corpusele (*cp*), a constituent of the attraction sphere (figs. 1, 2, 4, 6). In reality, on transverse section one sees a diplosome, two granules superposed, of which only one is visible in a tangential section. Generally central, they may become eccentric and even reach the periphery of the field. The corpusele is surrounded by a small, clear area, the medullary zone of the attraction sphere of E. Van Beneden, which is itself encircled by a darker cortical zone (fig. 4).

The diplosome also exists in the small fields of the indifferent mosaic covering the future lesser ridge (figs. 1 and 2). When differentiation occurs and is completed the diplosome persists within the sensorial fields, where it becomes peripheral, occupying the outer or lateral part of the round apex of the hair-cell. It is always surrounded by a small, clear medullary area (*cp*, fig. 5) beyond the dense, intensively stained circular central plate from which proceed the hairs. This superficial plate is considered by N. Van der Stricht (1908) and Held (1909) as a cuticular product of the cytoplasm. Series of preparations of the cochlea of young cats, fixed by osmic vapors or 1 per cent osmic acid followed by treatment

by other agents, show the presence of innumerable mitochondria and chondriomites throughout the cytoplasm of the sensorial cells. Near the free surface of these elements the mitochondria increase in number and are in very close contact; on the surface they form a plate which is more or less homogeneous, as if the granules were fused together. In successful thin preparations the mitochondrial nature and the granular structure of the superficial plate may be observed. This proves that cuticular formations belonging to the first series mentioned above may be of mitochondrial origin, but in addition it is a striking proof of the mitochondrial nature of the acoustic hairs formed by this plate. F. Spee (1901), Held (1902), and N. Van der Stricht (1908) have described the central corpuseles of the hair-cells, and the last two authors are agreed that these diplosomes do not take part in the formation either of the cuticular plate or the hairs. According to N. Van der Stricht, the superficial central corpusele of the hair-cells (crista and macula acustica) forms a flagellum; Held (1909) observes two flagella for each diplosome within all the epithelial cells lining the cochlear duct. The superficial central corpusele shows a flagellum prominent on the surface and on the deep face a flagellum directed into the protoplasm towards the nucleus.

The diplosomes within the irregular polygons of the sustentacular fields of the organ of Corti are repelled into the enlarged axial or inner portion of the inner supporting cells (fig. 4), into the enlarged lateral portion of the phalanges of the outer pillars as observed by N. Van der Stricht and Held. In the phalanges of the first and second rows of Deiters cells (and of the third in case of an additional fourth row) in the new-born dog they are divided into two central corpuseles, one of which reaches the axial segment and the other the lateral segment of the field. At this stage of development (figs. 4 and 5) the central corpuseles are not displaced in the small polygons of the inner pillars, in the third row of Deiters cells, and in the fourth when it exists.

What is more important in elucidation of the anatomical substratum of the membrana tectoria is the appearance of the terminal bars—the system of lines which separates all the polygons, the apices of the cells, of the superficial mosaic, and closes the intercellular spaces. These bars, described for all endothelia and columnar epithelia, represent a denser and superficial portion of the intercellular substance, the chemical composition of which is altered, for it takes up intensively various stains (such as iron hematoxylin) in the same manner as do the central corpuseles and the cuticular superficial plates of the acoustic cells. The size of the terminal bars varies according to the stage of development and the region. Originally thin, they remain thus in the region of the cells of Hensen and of Claudius. They enlarge slightly at the surface of the crista spiralis, but become much thicker on the greater epithelial ridge and between the constituents of the organ of Corti.

My preparations from pig, bat, dog, cat, and mouse enable me fully to confirm the results obtained upon bat (*Vespertilio noctula*) by N. Van der Stricht, who considers the membrana reticularis of the crista and macula acustica and of the organ of Corti as formed exclusively by a gradual enlargement of the terminal bars. In 1876, after fixation of material by silver nitrate which stained the inter-

cellular cement (Kittsubstanz) in black, Lavdowsky expressed the opinion that the membrana reticularis is formed by this metamorphosed substance.

Figure 8, a section tangential to the surface of the crista acustica in a newborn dog, shows a system of thick terminal bars (*tb*) between the smaller polygonal supporting fields (*suf*), each of which presents a central corpuscle and the larger more circular sensorial fields (*sf*), within which a dark central plate and an eccentric central corpuscle (*cp*) are visible.

Figure 9 represents a similar appearance of the crista acustica in an adult bat (*Vespertilio fuscus*). Here the bars (*tb*) are very much enlarged and extend over the greater part of the clear sustentacular fields, leaving uncovered only their central area (*suf*). The more or less circular openings (*suf*) of the membrana reticularis become smaller in figure 10, the crista acustica of an adult white rat, and are narrowest in figure 11, the macula acustica of an adult mouse. The much larger sensorial fields (*sf*) of these last three figures show the central dark cuticular plate from which proceed the hairs traversing these large openings of the membrana reticularis. The power of enlargement and extension of the originally thin terminal bars is fully demonstrated by these four figures, as also is the real origin of the fenestrated membrane derived from them.

As regards the origin of the membrana reticularis of the organ of Corti, figures 4, 5, and 7 are noteworthy. During the earliest stages in the process of development of the membrana tectoria the bars separating sensorial and supporting fields are rather thin, although much thicker than those visible in my preparations at the surface of the cells of Hensen and of Claudius, but they gradually enlarge, chiefly after the membrana tectoria is formed. In the adult organ of Corti one sees (fig. 7) well how the bars have become thicker everywhere and are enlarged most between the first and second rows of outer hair-cells and in such a way that between these two rows, and again between the second and third row, there is to be seen a system of lines alternately thin and thick (*tb''*), but relatively thicker in the latter situation. Finally, along the row of inner hair-cells between two neighboring sensorial fields (fig. 7', *ih*), there is a small, dark veil hiding the apices of the inner supporting cells. This originates as an extension of the terminal bars, and I was able to see similar figures and superficial veils in the organ of Corti of adult rats and dogs. The development of this veil is another striking proof of capacity for extension over neighboring cells possessed by the terminal bars. It may be recalled here that the existence of a small plate or a prolongation of the head of the inner pillar has been mentioned previously by Retzius (1884) and Held (1902). According to Retzius, who did not recognize the inner supporting fields, it extends between the apices of two inner hair-cells. Held, who terms this prolongation a rostrum (Sehnabel) or a bill, describes it as spreading over a small outer zone of the inner supporting cells. I presume that this *rostrum* is a part of the superficial veil which I have described as derived from the terminal bars, but which these two investigators did not recognize.

During the development of the membrana tectoria the terminal bars, as already stated, possess the power to grow and thicken upon the surface of the greater

epithelial ridge and even of the crista spiralis. This property may result in further alterations. At certain places the bars show a tendency to split longitudinally into two parallel lines, the clear space between which is bridged across, as in the case of so many intercellular spaces between epithelial cells. According to N. Van der Stricht (1908), this process of longitudinal splitting of the bars occurs regularly in the course of development of the membrana reticularis covering the crista and macula acusticæ. It also takes place during the formation of the membrana limitans olfactoria (1909). The power of extension of the bars over the apices of neighboring cells I will discuss in a later chapter.

The superficial epithelial mosaic of the cochlear duct has been described by Lavdowsky (1876) and Retzius (1884). Both investigators used silver nitrate as a fixing agent and thus stained the intercellular cement black. Vernieuwe (1905), after staining the terminal bars intensively blue by iron hematoxylin, first recognized the true nature of these elements and their special chemical composition. He observed the indifferent mosaic of the greater ridge, and since the number of fields and the number of nuclei deeply situated are approximately the same, he concluded that all the cells reach the surface, and consequently that this thick epithelium ought to be considered as a simple columnar epithelium. In 1902 the terminal bars on the surface of the adult organ of Corti had already been described by Held; in 1908 N. Van der Stricht and in 1909 Held studied them in the embryonic and adult cochlea on the surface of the indifferent and sensorial epithelium.

CRISTA SPIRALIS, LIMBUS SPIRALIS, HABENULA DENTATA, HABENULA SULCATA.

Former authors, including Huschke (1832) and Corti (1851), mention the existence of two regions in the surface of the crista spiralis. One, lateral—the zona dentata or sulcata, near the vestibular lip of the sulcus spiralis—displays a series of elongated protuberances more or less parallel—the teeth of Huschke, separated by furrows within which Corti had previously noted vestiges of nuclei. Another region, axial, near the attachment of Reissner's membrane, exhibits prominent "warts," "swellings," or papillæ, and may be termed the zona papillaris.

The crista is formed by connective tissue which was regarded by Hensen (1871) as cartilaginous, or as intermediary between cartilage and connective tissue. Gottstein (1870) and Waldeyer (1872) considered it, likewise the teeth, as osteoid substance and calcified. Hensen (1863) and Kölliker (1867) described the teeth as a product between and derived from the superficial epithelial cells, but Boettcher (1869), Waldeyer (1872), Denis (1901), and Vernieuwe (1905) among others, demonstrated that they are formed by a proliferation of the subjacent connective tissue between these elements. What becomes of these epithelial cells during and after this proliferation? Boettcher (1869) and V. Winiwarter (1870) noticed rows of nuclei without cytoplasm within the furrows separating the teeth, and Winiwarter described a kind of superficial mosaic without nuclei. He stated it in the following terms: "Sehr eigentümlich ist die auf der oberen Fläche des Gehörwulstes mit stärkeren Vergrösserungen wahrnehmbare Epithel-Zeichnung, hervorgebracht durch feine, scharf ausgedrückte Contouren ohne Spur von Kernen." It is, of course,

the real mosaic figured and described later by Lavdowsky (1876), Retzius (1884), N. Van der Stricht (1908), and Held (1909). After treatment by silver nitrate, Lavdowsky notices on the *limbus spiralis* a layer of small endothelial cells devoid of nuclei, more exactly endothelial plates which are quite distinct from the subjacent cells located within the interdental furrows.

On the *limbus spiralis* of rabbit, cat, and man, Retzius, also using silver nitrate, states that the interdental cells situated within the furrows reach the surface and line by their superficial flat apices the prominences, the teeth, and the warts (Warzen). Hence in adult individuals is formed a complete cell mosaic, continuous with the cell-layer of the Reissner's membrane and of the *sulcus spiralis*. This statement is confirmed by N. Van der Stricht and Held, who used other fixing agents and stained the terminal bars between the apices of the epithelial cells and found a diplosome within each polygon of the mosaic.

It is to be pointed out that the last three authors, who accurately describe the superficial mosaic and its connections with the teeth and the primitive epithelial cells, do not give exact details of the location of the cell bodies and of the intermediary connective tissue, because they did not examine transverse sections of the *crista spiralis* at different stages in its development. There is no wonder that in many text-books of histology the description of the superficial elements of the *crista* is partly erroneous. I will content myself with referring to the *Histology of Stöhr* translated by Billstein, 1898. On page 380 one reads: "the surface of the *limbus* is covered by a simple layer of flattened epithelial cells." R. Krause, in the *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbelthiere*, O. Hertwig, 1900, Bd. 1, p. 118, expresses himself in the following terms: "Die anfangs kubischen Zellen werden ganz platt und bilden eine feine, endothelartige Membran, welche hier den *Ductus cochlearis* begrenzt." In the text-book of *Microscopic Anatomy*, by E. A. Schäfer, 1912, one reads on page 285 that the cells "are continued as a pavement-epithelium over the *limbus*." In 1909 Vasticar described very flattened polygonal cells at the surface of the teeth of Husehke.

In order to get a true picture of the structure of the *crista spiralis* at various stages in its development, it is necessary to compare tangential sections with the vertical, and to follow, step by step, the formation of the teeth of Husehke and the alterations in the epithelial cells. I will distinguish four stages in succession.

1. The first is represented in the second turn of the cochlea from a pig embryo of 93.5 mm. The columnar epithelium is separated from the subjacent connective tissue by a basement-membrane stained blue by Mallory's method and green by light green, and is in continuity with the much thicker epithelium which outlines both ridges. On vertical and somewhat oblique sections this basement-membrane is fenestrated and provided with small openings; small nuclei of connective tissue, cells are incorporated in its thickness, their axis parallel to the surface of the epithelium. Beneath the membrane exists an embryonic areolar connective-tissue consisting of cells the nuclei of which are stained red by Mallory's method or blue by iron hematoxylin, and surrounded by a very small cytoplasmic zone stained faintly red by fuchsin or rosy by Congo red; this zone is in continuity with prolongations

taking up the same stain. But around and between the cells one sees an alveolar system, on optical sections a network of blue (if stained by Mallory's method) or green (if impregnated by light green) collagenous sheets or filaments in continuity with the basement-membrane. Within the spaces of the reticulum sections of the protoplasmic prolongations of the cells are visible. The alveoli, large in the deep layers, become gradually smaller in the neighborhood of the columnar epithelium. The superficial epithelium is represented by a row of prismatic cells, the nuclei of which may be situated at various heights. Each cell contains a single nucleus and the intercellular spaces are closed by the terminal bars already mentioned.

2. The second stage is that of the beginning proliferation of the connective tissue between the epithelial cells. Figure 12, from a photograph of the second turn of the cochlea in a 127.0 mm. pig embryo, shows this process in vertical section. The basement-membrane (*bm*), more or less visible on the right toward the future zona papillaris, has disappeared toward the left near the sulcus spiralis (*cssp*), where the proliferation first begins and is always most advanced. There exist below the epithelium larger alveolar spaces, and the constituents of the membrane with the collagenous elements of the connective tissue extend between the bases of the epithelial cells in the form of dark intercellular filaments. At first sight this intraepithelial connective substance seems to be homogeneous and no transverse sections of bundles are perceptible. Upon careful observation, however, it shows very small spaces within which thin prolongations (*pr*) of connective-tissue cells are detected. Hence it must be recognized that from the first stage of proliferation cell-prolongations and collagenous walls or sheets penetrate between the epithelial elements. Tangential sections of the crista spiralis demonstrate that this proliferation is performed in such a way that the epithelial cells are pressed together in more or less parallel rows along the future zona dentata (fig. 13, *zd*). The axis of the cellular rows (*ep*) is also parallel to the surface of the limbus and is directed from the future vestibular lip toward the zona papillaris. No collagenous substance exists between cells of the same row. Figure 12 proves that neighboring epithelial cells of the greater ridge, which later will cover the sulcus spiralis (*cssp*), participate in this alinement and special arrangement of the epithelial elements. These very small parallel intraepithelial connective sheets (*ic*, fig. 13) represent, of course, the future teeth of Husehke, which in oblique tangential sections (*t*, fig. 14) are clearly in continuity with thicker subepithelial parallel septa of the same nature (*st*, fig. 14). Consequently, it is obvious that this intraepithelial arrangement in the form of teeth of Husehke is in the first place induced by a special and similar disposition of the subepithelial substratum.

3. The distinctive feature of the third stage is that the connective-tissue teeth are more or less as large as the interdental epithelial sheets. Vertical sections of the crista spiralis in a pig embryo of 127 mm. stained by Mallory's method show this fact (fig. 16). Each superficial epithelial cell (*ep*) seems to contain two or three nuclei (owing to the oblique direction of the section) and to be cylindrical; but the red-stained cytoplasm at its free surface is in continuity with a similar small superficial layer (*mer*), dark in the photograph, covering the intermediary

teeth (*t*) and representing the superficial mosaic, which is never invaded or traversed by the proliferating connective-tissue. The transverse diameter of some cell-bodies is the same at various heights, but in others it is slightly reduced and constricted near the surface.

Between the dark epithelial elements exists a clearer collagenous mass (*t*), deeply stained by aniline blue, within which are noticed darker filaments stained red. These are the prolongations of subjacent connective-tissue cells (*pr*). These connective interepithelial teeth of Huschke extend bodily into the depth (*st*) and are largely in continuity with the subepithelial substratum. Many preparations show this detail more distinctly.

Tangential sections at this stage are very interesting (figs. 17, 18, 19). Figure 17 shows a portion of the third turn of the cochlea in a new-born dog. Above one sees the superficial epithelial mosaic (*mcr*) like a veil formed by elongated polygons, the apices of the epithelial cells, which are separated by darker lines, thin terminal bars stained blue by iron hematoxylin. The long axis of these fields is perpendicular to the axis of the rather dense subjacent bands (*pb*) which represent cytoplasmic epithelial zones deprived of their nuclei because the razor has taken only the superficial segments of the cells. In the lower part of the figure the veil disappears and one notices nuclear bands (*nb*), the section of the deeper segment of the epithelial elements. These bands are more or less parallel in the zona dentata (*zd*), ramified and anastomosed in the form of a network in the zona papillaris (*zpp*). Figure 19 shows much better the reticulum of nuclear bands (*nb*) in a pig embryo of 127 mm. The zona dentata is cut more superficially and one sees its mosaic (*mcr*) and a part of the membrana tectoria (*mt'*). Figures 17, 15, and 18 demonstrate also that the neighboring epithelial cells of the greater ridge (*essp*), conspicuous by their larger and darker nucleus, join the epithelial elements of the crista spiralis in the formation of the cell-bands. The connective tissue between the nuclear bands represents the teeth of Huschke and consists of a clear collagenous mass and darker granules (*pr*), the transverse section of the prolongations of subjacent connective cells.

In areas of figure 17 and more clearly in figure 18 one sees other details. A system of regular parallel filaments crosses the teeth (*t*) transversely like intercellular bridges and connects two neighboring nuclear rows (*nb*). There seem to exist as many bridges as there are nuclei in one column. Preparations of pig embryos of 127 and 137 mm. display similar bridges and their regularity proves that they represent real structures. I think that they are thin cytoplasmic membranes persisting between the cells of two neighboring rows after their separation by the proliferating connective tissue. At this third stage of its development the subepithelial parts of the teeth of Huschke are marked on tangential sections by large, thick, dense, parallel, collagenous bundles, within which many cell prolongations are embedded.

4. The final or adult stage is characterized by the fact that the cytoplasmic epithelial sheets are very thin and constricted in their superficial non-nuclear segment, which is in continuity with the persistently intact superficial mosaic; they are much larger at the level of their deep nuclear segment.

The two vertical sections represented in figures 20 and 21 give a true picture of these conditions. The first is from a pig embryo of 190 mm., the second from a young dog of about 4 months. Compared with figure 16, they show that the teeth (*t*) and the interdental epithelial sheets (*ep*) become much longer, as seen in figure 20. The teeth enlarge superficially, and by compression the intermediary cytoplasmic sheets are mechanically reduced to a kind of membrane which remains in direct continuity with the superficial epithelial mosaic (*mcr*). This latter is stained a little darker than the thin cytoplasmic sheets. In the depth the teeth keep their previous size or enlarge very little between the nuclear portions of the sheets, but from there their transverse diameter rapidly increases toward the surface and toward the depth. On the contrary, the epithelial bands become thinner in both these two directions. At their base the teeth with their cell prolongations merge with the subjacent connective-tissue.

Figure 21 shows the different layers of the adult crista spiralis:

1. Superficially the membrana tectoria (*mt*).
2. The cytoplasmic mosaic (*mcr*) beneath the former.
3. The epithelio-connective layer formed by the teeth (*t*) and the interdental epithelial sheets (*ep*).
4. A subepithelial connective layer formed by a collagenous substance, including occasional spaces with stellate cells and a system of canals with cell prolongations (*pr*). These latter are visible in length, in great number reaching the surface of the teeth. The teeth of Huschke are real extensions of this subepithelial layer.
5. A deep layer where the fundamental substance is less abundant and the cells with their prolongations more numerous.
6. A periosteal membrane (*per*), which later will undergo ossification and form a bone lamella separating the crista spiralis from the subjacent nerve-fibers (*ner*).

My description of the connective tissue is for the most part similar to that of Vernieuwe (1905).

Tangential sections of the adult stage of the crista spiralis are represented by figures 22 and 23. The first is from a pig embryo of 190 mm., the second from an adult bat (*Vespertilio fuscus*). They show three different planes in succession. The first and most superficial is the mosaic (*mcr*), consisting of clear polygonal fields separated by rather thin terminal bars (fig. 23), which in figure 22 are split longitudinally and exhibit intercellular bridges. Figure 24 displays a similar veil from the cochlea of a young dog, but within the polygonal fields one sees a dark circular mass, a kind of plate which represents the attraction sphere (*sph*) formed by a central corpuscle (*cp*), a small, clear medullary zone, and a larger dark cortical zone. In the cochlea of the adult mouse I could notice the successive stages of division of this dark layer into two smaller dark plates. It is an unexampled and surprising process in the evolution of the sphere, the function of which in the cell completely ceases after the last mitosis during the earliest stages of development.

A second plane, cut by the razor a little more deeply, shows a system of dark bands, granular and non-nuclear (*pb*), which become nuclear in the still deeper

third plane (*nb*). They are more or less parallel in the zona dentata and anastomosed in the zona papillaris (*zp*, fig. 22). In preparations from the pig embryo they are stained rosy by Congo red, and in those from the bat they are faintly blue from iron hematoxylin, the nuclei being dark blue. The nuclear bands are larger than the cytoplasmic.

The teeth of Husehke (*t*) between these epithelial sheets are clear and homogeneous in figure 22, like the papillæ of the zona papillaris (*zp*). But in figure 23 the teeth are striated from the presence of long granular filaments stained faintly blue, the prolongations of subjacent connective cells. The fundamental substance of the teeth is stained rosy by Congo red.

Transverse compared with tangential sections permit one to conclude that in the adult crista spiralis the interdental epithelial sheets (lamellæ) are much longer than in previous stages and reach a deeper level in the subjacent connective-tissue, but that they remain in direct continuity with the superficial cytoplasmic mosaic, covering entirely the surface of the teeth and the papillæ. Beneath this mosaic their transverse diameter is the smallest, while in their deeper nuclear portion it remains practically the same as in the preceding stage or decreases a little. This elongation and thinning of the sheets is due to a broadening and mechanical pressure of the intermediary teeth.

The reduction in size and in number of nuclei of the epithelial lamellæ is shown much better by the tangential sections than by the transverse. This change, more apparent than real, may be imputed to their elongation in a direction parallel with the axis of the primitive cell and undoubtedly to the wide extension of the sheets during the increase in size of the crista spiralis. But comparing figures 22 and 23 (where long cytoplasmic bands separate two rather small nuclei) with figures 18 and 19 (where the neighboring nuclei are in close contact) it seems to me that during the development of the limbus spiralis an increase of the cytoplasmic mass occurs and can not be denied.

This consideration brings me to the important question of study of the epithelial lamelle of the crista spiralis; all authors describing vertical sections of this admit the existence of separated cells, the primitive epithelial cells. Indeed, figures 12 and 13 prove that during the first and second stages this view is correct, and figures 16, 20, and 21 seem to confirm it for the third and fourth stages. All sections tangential to the surface of the crista, however, show different figures. At the third stage (figs. 17, 18, 19) no boundaries between cell-areas can be detected either in the cytoplasmic or in the nuclear bands where the nuclei are very closely pressed together. This statement is also true for the fourth stage (figs. 22 and 23). Consequently these epithelial sheets must be considered as real syncytial masses formed by fused epithelial cells, which are separated only during the first and second stages of their development. This fusion is brought about by a mechanical factor, the compression from the broadening teeth. This multinuclear syncytium is unexampled in other organs, while here the primitive apices and central corpuseles of the epithelial cells persist intact, the boundaries being marked by the terminal bars. Retzius (1884) and N. Van der Stricht (1908) refer to similar figures, but

the former author, though his illustrations show undivided nuclear cytoplasmic masses, speaks of "interdental cells;" the second mentions "nuclear bands." Neither describes the syncytial nature of these formations.

As a matter of fact, in many preparations some exceptions to this rule may be found with spaces between the neighboring cells. I do not refer, of course, to figure 20, where the razor cut the teeth vertically just at the edge of the vestibular lip and struck the epithelial cells of the subjacent sulcus spiralis, an unchanged columnar epithelium (*essp*); I refer to figures which may be observed in the middle of the limbus. Their existence proves that when the mechanical pressure is weak fusion does not occur, or when it diminishes in adult stages boundaries may reappear.

Do the numerous nuclei of this special kind of syncytium represent elements of the primitive isolated cells? Or are new nuclei formed at the time of fusion of the cell-bodies or afterward? As a matter of fact, after the first stage described above, no mitoses occur within the epithelial cells. On the other hand, some preparations seem to confirm the idea that occasionally nuclei undergo nuclear amitosis, increasing in size and elongation, and exhibiting direct division into two smaller daughter nuclei by a process of constriction.

Retzius was able to investigate this question. His figures represent the superficial mosaic with terminal bars stained black by silver nitrate; below each polygon he notices a single nucleus, hence he asserts that there exist as many apices of cells as nuclei, although near the vestibular lip some fields contain two nuclei which he explains as due to the fact that the supplementary elements belong to the sulcus spiralis. But by a careful examination of the figures of Retzius I can sometimes count three nuclei beneath two superficial fields at a short distance from the vestibular lip (his figure 1, plate 24) and also in the zona papillaris.

If, in preparations similar to that displayed in figure 17, I compute the number of superficial fields along one row of polygons of the zona dentata and the number of nuclei of one nuclear band, I find that I obtain as a rule the same number (about 12); but there are some exceptions where there are one or two nuclei more than fields. I am inclined, consequently, to believe that some occasional nuclei of the primitive epithelial cells undergo the process of amitosis during the second or third stage of development of the crista spiralis.

Finally, I think that it is worth while to emphasize the fact that furrows mentioned by many authors between the prominences of the teeth, within which (according to v. Winiwarter and others) nuclei or remains of epithelial cells are located, do not exist at all. There are large interdental spaces completely filled by the epithelial syncytial lamellae. In the adult organ the deep portion of these spaces is broad, but the superficial gradually becomes very narrow at the level where the cytoplasm is in continuity with the superficial mosaic. Superficial pits between the teeth are either exceedingly small or are lacking; if they do exist they are covered by only a part of the mosaic. The fused epithelial cell-bodies are embedded between the teeth in such manner that I can not agree with Retzius (1884, p. 345) when (in describing these conditions of the adult man) he states: "Diese Epithelzellen welche sich beim Embryo als eine cylinderzellen Schicht anlegten sind also noch beim

Erwachsenen als Cylinderzellen erhalten, nur sind sie reihenweise von einander durch die von unten her emporwachsenden Vorsprünge getrennt worden."

Retzius adds that these cells are actually so adherent as to remain attached to the membrana Corti when it is torn away from the underlying tissues and that even after maceration they still remain connected with the membrane! Such incidents may occur only when the first or second embryonic stage persists. In this respect I am able to state that large areas of the zona papillaris from cats 1 to 11 days old are covered by a cubical epithelium free from connective-tissue, while other parts of the same region and the zona dentata show the structure of the adult stage. According to Gottstein (1870) and Waldeyer (1872) half the surface of the crista spiralis, next to the attachment of Reissner's membrane, remains covered by a continuous layer of epithelial cells. Of course, such elements may become detached by maceration or by stripping off the membrana tectoria.

DEVELOPMENT OF THE MEMBRANA TECTORIA.

ON THE SURFACE OF THE GREATER EPITHELIAL RIDGE.

The process of genesis of the membrana tectoria is conspicuous mainly at the most active portion of its anatomical substratum—that is to say, at the surface of the greater epithelial thickening. Tangential sections must be obtained, since they exhibit the best figures, as demonstrated by figures 25 and 26. One observes the superficial mosaic (*mg*) removed by the razor from small areas (*mt'*), where the recently formed part of the membrana tectoria is visible. At *mt'* exists a kind of pale mosaic of another nature, reproducing that of the subjacent anatomical substratum. The terminal bars are replaced by larger dense lines and the apices of the cells by paler, more circular areas. The lines exactly overlie the system of terminal bars which is cut off, and their substance is not only in close contact with the bars, but is in continuity with them at the periphery of the small islands. The pale, more fluid substances in the mazes of this network overlie the polygons of the mosaic, the cytoplasmic apices of the epithelial cells, and are also in continuity with them. Hence the compact lines must be considered as produced by the bars and the content of the mazes by the superficial cytoplasm.

By Mallory's method the lines are stained blue, the bars red; by the use of iron hematoxylin and Congo red the former are rosy, the latter dark blue; after iron hematoxylin and light green the first are intensely green and the second dark bluish. Consequently, the chemical composition of the line must be regarded as different from that of their generating substratum.

The cochlea of a pig embryo of 93.5 mm. fixed by the uranium nitrate method of Ramon y Cajal shows in some places (fig. 27) the terminal bars (*tb*) and the dense part (*tb'*) of the superficial membrana tectoria stained black. The compact lines (*tb'*) are thinner than the bars and situated in a different plane; hence they are not quite in focus. The result of this treatment affords another striking proof of the real origin of the lines and of a chemical composition more or less similar to that of the bars. The lines (*mt'*, figs. 25 and 26) are larger than the bars and at first sight

seem to be homogenous and structureless; but on careful examination (mt' , figs. 25', 26') they look double, as if split longitudinally into two parallel thin lines severed by a clear space which is at times bridged across, the bridges being immersed in a kind of intercellular substance.

A tangential section through all the layers of the membrana tectoria (mtg) over a large extent (fig. 28) proves that the same structures are visible everywhere, the mazes of this kind of network becoming a little smaller toward the surface of the membrane (on the right side of the figure) than in the vicinity of its inferior side, next to the mosaic of the greater ridge. Nowhere can there be seen transverse sections of filaments or of fibrils. Hence the reticulum in figure 28, and mt' , figures 25 and 26, must be considered as the optical section of a system of walls, of membranes surrounding cylindrical or prismatic tubules filled with a pale fluid. In other words, the membrana tectoria is formed by a system of cylinders or prisms consisting of a dense outer wall derived from the terminal bars and of a contained portion, the more fluid part, derived from the cytoplasmic apices of the epithelial cells.

This view is confirmed by vertical sections through the greater epithelial ridge, as represented by figures 29, 30, and 31, taken from the cochlea of a pig embryo of 95 mm. These show the cylinders or prisms lengthwise as double lines (cy) and cut across as circular or polygonal fields (cy'). Their transverse sections in figures 30 and 31 are quite similar to those in figures 28, but the longitudinal sections (cy) demonstrate better that between the cylinders exists an interprismatic clear substance with delicate structures, within which elements like bridges can be noticed. Figure 29 shows most clearly that the young membrana tectoria consists of a basal clear layer and one more superficial, darker and more compact, the walls of the cylinders being denser near the surface and their diameters being a little smaller, with an intermediary substance less abundant. Finally, the base of each prism is obviously in continuity with one of the slightly prominent apices of the epithelial cells; but these vertical sections can give no sure knowledge concerning the origin of the constituents of the membrana tectoria. Therefore, tangential sections are needed.

We shall see that the interprismatic substance undoubtedly exists in the membrane of adults; hence it is not an artificial product, the result of shrinkage. It is derived from the terminal bars which may split longitudinally into two thinner parts connected together by short bridges. The primitive bars close the subjacent intercellular spaces and separate the intercellular cement from the interprismatic substance, which must therefore be considered as derived from the bars themselves.

Some authors, Coyne and Cannieu (1895), Hardesty (1908), Held (1909), and Prentiss (1913), have drawn and described figures similar to those in my figures 25, 26, mt' , and also regard them as representing the first stage of the developing membrana tectoria. Hardesty and Held consider these figures as a network of filaments derived from the superficial cytoplasm of the epithelial cells and forming the fibers of the adult membrane. Hardesty (1915, p. 60) states:

"In the production of the tectorial membrane each cell of the greater epithelial ridge may contribute an average of 25 fibrils to the membrane. Each fibril seems to show a

slightly elongated enlargement at its junction with its cell. In the region of the immediate surface of the ridge the interfibrillar matrix does not appear as abundant or so completely produced as in the older body of the membrane."

In many figures Hardesty and Prentiss represent the bars, but do not describe them.

Held computed 33 to 38 fibrils per each 100μ of the surface of the greater ridge. They are stained red and visible as red granules on sections tangential to the superficial mosaic, each polygon displaying some of them (his figure 3, guinea-pig). Nowhere else does this author mention transverse sections of these elements in the developed membrane, although occasional figures seem to show them. He describes the terminal bars everywhere, but does not attribute to them any importance in the genesis of the membrane. However, investigating the origin of the cupula of the surface of the macula acustica in the rabbit, he states that the reticulum, representing the first stage of development of the cupula, remains attached to the apices of the sustentacular cells by delicate filaments, "wobei sie oft nicht in mitten der Zellfläche, sondern mehr einer Schlussleiste zu sich anheften" (p. 265). He mentions further, beneath the cupula, special filaments in connection with the surface of the sensorial epithelium, and adds: "merkwürdigerweise sind diese besonderen Fäserchen mit den Schlussleisten seitlich verbunden, oft in reicher Zahl von diesen abgehend, welche eine Haarzelle umgreifen." Finally, referring to the filaments of the tectorial membrane in the hen, the chicken, and the pigeon, he states that they "an den freien Flächen der Stützzellen und oft dicht an den Schlussleisten sich anheften" (p. 275). In spite of these statements, Held accords no importance to the terminal bars in the development of the membrana tectoria.

Held gives further details of the anatomical substratum of the membrane. In the rabbit he observes a special homogeneous border on the surface of the epithelial cells, within which the diplosomes and the terminal bars are inclosed. It is his "Randsaum" and undoubtedly my superficial mosaic. But above it appears another very thin striated border, his "Decksaum," and on page 203 he states: "Die Vorstellungen die ich nur auf Grund dieser Beobachtungen gebildet habe, ist, dass der durch den Rand- und Decksaum ausgezeichnete Epithelbezirk die erste Bildungszone der Cortischen Membran ist."

Figure 32 represents a portion of the membrana tectoria and the superficial mosaic of the subjacent greater ridge in the vicinity of the future sulcus spiralis (*ssp*) partially free and detached from the membrane. It displays some structures very interesting in relation to the fibrillar origin of the membrana tectoria as described by Hardesty and Held. While some fields of the mosaic are clear (*f*), others are covered by a dark veil (*f'*) or a granular veil stained like the bars. Indeed, many preparations prove that these latter are able to enlarge and extend over the neighboring polygons. But some structures, real filaments (*f''*) derived from the bars, incline and join together over the polygons more superficially where they (the said structures) generate the dense part of the cylinders. If these fibers persist in the walls of the prisms one would have to recognize the fibrillar structure of the walls, but my preparations do not allow me to ascertain with certainty if this be so.

The same figure shows also the extent of shrinkage often produced by fixing agents through artificial distention of the more fluid part of the recently formed layer (*mt'*) of the membrana tectoria.

Coyne and Cannieu describe the membrana Corti as by no means formed by a homogeneous clear substance with dense fibrils, but by membranes or sheets ("cloisons") of a special nature circumscribing polygonal cavities which form a network in perpendicular sections. "The surfaces of junction of these membranes thickened at the angles of the reticulum" (p. 280) represent the alleged fibers; and on page 285 they add: "these sheets circumscribe polygonal cavities which gradually become narrower from the organ of Corti toward the prominence of Huschke."

My results upon the development of the membrana tectoria are chiefly comparable with those of Prentiss. On page 442 this author states:

"To sum up the development of the membrana previous to fetuses of 15.0 cm., we may say that it is a cuticular organ with a definite though irregularly chambered structure which is secreted between, and at the ends of the cells composing the basal epithelium of the cochlea."

In his conclusion Prentiss adds:

"In sections through the axis of the cochlea the membrana has a striated or lamellated appearance. . . . In sections perpendicular to the lamellæ the structure of the membrana is that of a reticulum with thickenings at the angles of the meshes. It is therefore neither lamellar nor reticular but a chambered structure or 'honeycomb' of hollow tapering cuticular tubes or chambers normally filled with a fluid resembling the endolymph. The bases of chambers during development rest between the ends of the epithelial cells."

The main difference between the results of Prentiss and my own is that according to my investigations there may exist an interprismatic substance among the chambers, and that the walls of the cylinders are produced by the terminal bars which were not recognized by Prentiss, while their content alone is formed by the cytoplasmic apices of the cells.

ON THE SURFACE OF THE-LESSER EPITHELIAL RIDGE.

Figure 28 displays a section tangential to the surface of the organ of Corti, the neighboring greater ridge and a segment of the crista spiralis between the second and third turn of the cochlear duct in a new-born dog. *Ih* and *oh'*, *oh''*, *oh'''* show respectively the row of inner hair-cells and the three rows of outer hair-cells. Two hair-cells, *oh^{iv}*, belong to an interrupted fourth row of outer sensorial elements. This segment of the section and another (*mg*, the superficial mosaic of the greater ridge) are not in focus; therefore they are blurred, but the structures of the membrana tectoria are plainly visible on the right of the figure. In continuity with the rows of outer hair-cells one sees three different, more superficial planes *s'*, *s''*, *s'''*. The first (*s'*) displays horseshoe-like elements, the bases of the acoustic hairs; they occupy more or less the center of clear areas separated by a system of darker, thick, longitudinal lines (*l*) and of thinner transverse lines (*l'*). Some of these lines are double and short bridges connect the two halves. The clear areas undoubtedly correspond to the sensorial round fields of the outer hair-cells and overlie their

apices and the pale fluid must be considered as derived from this cytoplasmic substratum. The longitudinal, thick, irregular lines overlie the apices of the supporting fields better visible in figure 5, *op, d', d'', d'''*, where the terminal bars between two neighboring hair-cells of the same row seem to form a single thick line; while the thinner transverse lines overlie those bars, also irregular, visible between the different rows. Hence the longitudinal and transverse lines of the first superficial plane (*s'*) should be considered as derived from the subjacent terminal bars—that is to say, from the real membrana reticularis of the organ of Corti.

I believe, consequently, that I am justified in drawing the conclusion that the membrana tectoria is formed at the surface of the organ of Corti by the same process as that observed on the surface of the greater ridge. The differences in appearance of the figures are induced by corresponding differences in the anatomical substratum.

The second more superficial plane is also very instructive. It consists of two fields (*s''*) identical in size with those of the preceding (*s'*). Their longitudinal lines (*l*) are undoubtedly double and the two neighboring halves are connected by short bridges. The transverse lines can not be recognized and a delicate pale network is visible within the clear areas. The structures of this plane resemble those of the third superficial plane (*s'''*), where the longitudinal lines have also disappeared, and the constituents are the same as those of the neighboring membrana tectoria belonging to the greater ridge (*mtg*).

What is the significance of the pale network appearing within the clear spaces of the second plane (*s''*)? Two different explanations may be given. The network represents the most superficial portion of the membrane formed in the early stages of its development, before the appearance of the hair-cells, when the subjacent mosaic is formed by undifferentiated polygons (figs. 1, 2, *ml*). Figure 33, from a pig embryo of 150 mm., shows the membrana tectoria recently produced (*mt'*) on the surface of the second and third rows of Deiters cells (*d'', d'''*) and the third row of outer hair-cells (*oh'''*). One sees clearly (*mt'*) a series of pale round fields surrounded by very thick bands which, with similar lines around smaller areas, reproduce more or less the subjacent membrana reticularis of the lateral part of the organ of Corti. This figure proves that in the course of development the structures of the tectorial membrane change here greatly, as do those of the subjacent membrana reticularis, and that the differences between its first (*mt*) and its later-formed layers (*mt'*) become gradually more pronounced. I am thus induced to accept this first explanation.

A second explanation would bring me to consider this delicate reticulum as derived from the bars in such way that the dense part of the membrana tectoria directly formed by them and covering them may grow and extend over the apices of neighboring cells and there give rise to structures like those on the crista spiralis. I will refer to this question later.

Held (1909) describes a system of thick fibers derived from the apices of the sustentacular cells of the organ of Corti and chiefly from the first and second rows of Deiters cells; these filaments he terms "Haftfasern der Lamina reticularis

externa," adhering or attached fibers. They take origin from the phalanges by two limbs:

"Ein innere Schenkel ist von einem äusseren bei diesem zweispältigen Ursprung der Hauffasern der I und II D. Zellen zu unterscheiden, von denen sich jeder aus einer Summe von Fibrillen sammelt, welche auf die beiden entgegengesetzten und breiteren Ecken der Phalangen-platten hauptsächlich verteilt sind, wenn auch eine geringe Anzahl auf dem schmäleren Mittel stehen kann" (p. 221).

The double lines or double filaments of Held's figure 15 and the coarse fibers in his figure 16 are undoubtedly the same elements as my lines *l*, figure 29, which I regard not as fibrillar, but as bands or walls, homogeneous like the subjacent membrana reticularis.

It may be pointed out that, with N. Van der Stricht, I consider the membrana reticularis of the organ of Corti as fenestrated and constituted by a system of enlarged terminal bars separating openings within which the apices of the sensorial and of the supporting cells are located. Held (1904) and others describe the apices of the Deiters cells as a constituent of this membrane.

The membrana tectoria of the kitten deserves special mention. Fixed by osmic acid and Bouin's fluid or by trichloroacetic acid it shows on its lateral edge a regular series of coarse filaments, the thickness of which equals the diameter of the apices of the third row of Deiters cells. Upon each of these apices is adherent a thick, solid, long cylinder, a real cramp (un crampon), even where the entirely free part of the membrana tectoria is detached from the organ of Corti. I am not able to determine if these attachments persist in adult animals. The *cramps* mark clearly the lateral boundaries of the membrane and of its anatomical substratum. I have not, so far, had the opportunity of investigating the earliest stages of their development, since I have at present no embryonic cat material available. It seems to me that Retzius (1884) observed these elements in the cochlea of the cat, since he described, in the *Randfasernetz*, "glänzenden parallel neben einander von innen unten nach aussen oben verlaufende Fasern, welche sich am Ausserrande zur einem Randstrang sammeln."

ON THE SURFACE OF THE CRISTA SPIRALIS.

All authors agree that the crista spiralis takes only a slight part in the development of the membrana tectoria. According to my investigations, the alterations of its superficial mosaic in the earliest stages of its activity are essentially the same as those described for the greater epithelial ridge, although thinner terminal bars and larger cytoplasmic fields result in some differences. Figure 28 shows that the most superficial layer of the tectorial membrane, first developed and visible on the right of the figure (*mtr*), is formed by a system of larger fields and thinner intermediary membranes than those belonging to the neighboring segment (*mtg*) of the greater ridge. But in a layer a little deeper and more to the left the wide areas disappear, because a kind of delicate network covers them. Finally, still more to the left, the membrana tectoria of the crista spiralis exhibits the same structures as those of the greater epithelial thickening.

Figures 19 and 34, from a pig embryo of 127 mm., illustrate better the appearance of these formations. The two tangential sections are a little oblique and involve four successive planes. The first belongs to the nuclear and cytoplasmic layer of the zona papillaris (*zp*); the second and more superficial shows the superficial mosaic (*mcr*) of the zona dentata; the third exhibits wider areas (*mt'*) enlarged by the fixing agents. Some of these areas contain only homogeneous clear fluid, obviously a product of the superficial cytoplasm; they are separated by thick, coarse lines as dark in the figures as the much thinner lines belonging to the subjacent mosaic (*mcr*), the real terminal bars, although in my preparations they are but faintly stained by Congo red. This dense part of the tectorial membrane more recently formed (*mt'*) is derived from the bars and is in direct continuity with a delicate secondary network of the same nature (and therefore of the same origin) covering some of these clear areas. Finally, a fourth quite superficial plane (*mt*) represents the older part of the membrana tectoria and its constituent structures, but is more compact and reminds one of the structures in the membrane produced by the greater ridge.

The vertical sections, figures 14, 20, and 21, illustrate also the fact that the tectoria membrane (*mt*) on the surface of the crista spiralis is formed by a dense part, the uninterrupted walls between small cavities or chambers filled by a clear fluid. On the right of figure 20 the dense part is marked by long lines which are in continuity with similar constituents or cylinders belonging to the free membrane overlying the sulcus spiralis.

The features just described force me to conclude that at this stage of its development the membrana tectoria of the crista spiralis is formed by a system of chambers or cylinders with a fluid content derived from the superficial cytoplasm of the epithelial cells, and by denser walls produced at least in part by the terminal bars. Indeed, as already stated above, during the development of the membrana olfactoria limitans, of the membrana reticularis of the crista and macula acusticæ, and of the membrane covering the apices of the inner supporting cells in the organ of Corti, the substance of the bars extends over the neighboring fields and may form a kind of delicate network which gives rise to secondary structures of the same nature and of the same chemical composition as those of the bars.

In the case of the developing tectorial membrane the primitive bars generate on their surface a coarse primary network of a different chemical composition, the large meshes of which contain a secondary, more delicate reticulum of the same nature and of the same chemical composition. This secondary network is derived at least in part from the primary, perhaps in part also from similar cytoplasmic structures. To what extent the apices of the epithelial cells take part in the genesis of this delicate reticulum I am no more able to determine than Alice Thing has been (1917) in regard to a similar secondary network generating the fundamental substance of the zona pellucida in the turtle.

Many series of preparations of adult and embryonic stages showing features similar to those of figures 14, 19, 34, 20, and 21, prove that a great number of cylinders of the free membrana tectoria reach and are connected with one cytoplasmic

polygon of the superficial mosaic covering the crista spiralis. Each of them is for the most part derived from one polygon of the mosaic of the greater ridge, but also somewhat from a mesh of the secondary network covering one polygon of the crista spiralis, which (according to all investigators) represents the least active segment of the anatomical substratum. But it is impossible to give even an approximate estimate of the exact parts played by these two substrata.

I have been able to describe the genesis of an interprismatic substance between the prisms produced by the greater ridge, but the genesis of similar spaces between the portions of cylinders formed by the crista spiralis and by the membrana reticularis of the organ of Corti is still obscure. If further investigation should confirm the existence of this kind of cement for the first segment and the lack of it in the other two segments, this distinctive feature might enable other investigators to determine accurately the parts of the prisms derived from each of the three segments of the anatomical substratum.

STRUCTURE OF THE ADULT MEMBRANA TECTORIA.

Most authors distinguish three segments in the adult tectorial membrane: a thin, innermost axial segment covering the crista spiralis; an outermost lateral, derived from the lesser ridge; a thick middle segment produced by the greater ridge according to the investigations of: Henle (1866), Löwenberg (1868), Boettcher (1869), v. Winiwarter (1870), Gottstein (1870), Hensen (1871), Lavdowsky (1876), Nuel (1878), Retzius (1884), Barth (1889), Dupuis (1894), Coyne and Cannieu (1895), Held (1909), and Prentiss (1913).

The middle segment is striated and the striations incline from the vestibular lip toward the organ of Corti, the inclination being due to the existence of fibers or of fibrils as stated by Hensen (1863), Henle (1866), Boettcher (1869), v. Winiwarter (1870), Lavdowsky (1876), Nuel (1878), Tafani (1882), Retzius (1884), Ferré (1885), Ranvier (1889), Barth (1889), Ayers (1891), Dupuis (1894), Hardesty (1908), Held (1909), and Vasticear (1909). According to Coyne and Cannieu (1895), Shambaugh (1907), and Prentiss (1913), they are due to the presence of lamellæ. Most authors agree upon the presence of a homogeneous fluid between these constituents, though Retzius denies it.

The inner segment is described by Henle (1866), Boettcher (1869), v. Winiwarter (1870), Hensen (1871), and Nuel (1878) as a more or less homogeneous but fenestrated membrane provided with openings. Gottstein (1870) and Lavdowsky (1876) regard it as structureless and without openings. Retzius (1884), Barth (1889), and Dupuis (1894) consider it as formed by thin radiating fibrils which according to Held are collected in bundles separating openings. The outer segment, as stated by Henle (1866), Boettcher (1869), v. Winiwarter (1870), Gottstein (1870), Nuel (1878), Retzius (1884), Dupuis (1894), and Held (1909), consists of a network of anastomosed fibrils, filaments, or hyalin bands. Hardesty (1908) describes an accessory tectorial membrane as formed by two sets of fibers crossing at an acute angle.

Prentiss (1913) subdivides the membrana tectoria into the following zones:

1. A thin, structureless zone of the inner portion of the labium vestibulare.
2. A second thicker zone of flattened horizontal chambers over the outer portion of the labium vestibulare.
3. A still thicker zone of chambers curving downward and outward, unattached, over the sulcus spiralis.
4. An outer zone thickest in the upper turn, with chambers trending downward, outward then inward, largely attached to the cells of the spiral organ and probably normally and wholly attached in this manner.

Many investigators describe a superficial layer in some regions of the membrane, the superficial network of filaments, *Fadennetz* of Löwenberg, who mentions its existence throughout the two-thirds of the outer segment. Its existence is confirmed by Hensen (1871), Boettcher (1872), Retzius (1884), Dupuis (1894), Spee (1901), and Held (1909) as extending farther towards the inner segment. Spee and Held recognize that it reaches the attachment of Reissner's membrane and forms the inner zone of the tectorial membrane.

According to the process of histogenesis, the adult membrane is formed throughout its entire thickness and breadth by a system of cylinders or prisms separated by a pale and homogeneous fluid which seems to be lacking in the most superficial and densest layer. Figure 35, from an adult rat, represents a tangential section of the outer segment (*mtl*) and the greater part of the middle segment (*mtg*). At first sight the figure shows a system of double lines, the longitudinal axial section of the cylinders (*cy*). The intraprismatic liquid seems to be a little darker than the more abundant interprismatic fluid. One sees, moreover, other single, often darker lines, tangential sections of cylinders (*cy''*). At the ends of some obliquely cut cylinders the double lines join together and are in continuity with a single line. On the left of the outer segment (*mtl*) and in the middle segment (*cy'*) the prisms are pressed together and their transverse section is visible in the form of small polygons, between which the interprismatic substance seems to be lacking.

In my preparation the segment of the membrana tectoria photographed (fig. 35) is in continuity with its inner segment on the surface of the crista spiralis. This axial portion exhibits the same structures, but the prisms are in closer contact, while the interprismatic fluid is less abundant.

The superficial network of Löwenberg and similar structures of the outer and inner segments are represented, according to my preparations, by more compact parts of the membrane, the interprismatic substance of which is less abundant or lacking, so that a real chambered structure (Coyne and Cannieu, Prentiss), or, in tangential sections, a kind of network appears; but the walls of the chambers or the filaments of the reticulum represent the transverse section of the membranes of neighboring cylinders. Figure 35 displays these longitudinal prisms on the right side of the outer segment, while their apices are cut transversely, pressed together, on the left.

Figure 23, from an adult bat, exhibits a small portion of the middle segment of the tectorial membrane within which at least three similar longitudinal sections (*cy*) of the cylinders and some obliquely tangential (*cy''*) can be noticed.

In addition to these two figures, 23 and 35, of adult stages, I give another, similar, figure 36 and 36', from a young dog, and one, 37, from a pig embryo 150 mm., with similar structures, longitudinal (*cy*), obliquely tangential (*cy''*), and transverse sections (*cy'*) of prisms. They are larger and the intraprismatic and interprismatic fluid is more abundant. As a matter of fact, in some instances in these figures, instead of prisms with fluid content, there seem to be present transverse sections of solid filaments, and in transverse sections (*cy'*) darker granules like sections of fibers within the walls of the cylinders. Further investigation is wanted to decide if these elements correspond either to real structures or to products of the shrinkage induced by fixing agents, and if some delicate fibrils or bridges may persist within the pale intraprismatic or interprismatic fluid.

From this description I must conclude that the adult membrana tectoria is formed throughout its extent by prisms or cylinders consisting of an outer dense membrane or wall and of an axial clear fluid. They are separated by a more fluid intermediary substance, which may be lacking in some places upon the surface of the middle segment, but chiefly of the other segments, while a wall common to two neighboring prisms separates their pale content. This superficial layer is the densest and formed earliest in the course of development. Fixing agents, by reason of the consequent shrinkage, seem to have a great influence on these structures and induce many alterations.

The results of these investigations permit me to emphasize the analogy of structure and origin of the membrana tectoria and enamel of teeth. Both organs are cuticular formations derived from a columnar epithelium, and their immediate anatomical substratum is represented in the case of the former by the superficial mosaic of the cells, and in the latter instance by a basal mosaic, the cytoplasmic polygons of which are also separated by terminal bars according to the investigations of Cohn (1897). Both are formed by a system of prisms, each produced from one cell. The axial, more fluid part of the prisms of the tectorial membrane is derived from the cytoplasm and the outer denser wall from the terminal bars, which at the same time give rise to an interprismatic fluid. The solid calcified prisms of the enamel are produced by the cytoplasmic base of the ameloblasts, while the part taken by the terminal bars in the origin of the calcified cement has not thus far been demonstrated. It should be pointed out that one epithelial cell of the crista spiralis, and perhaps of the organ of Corti, may produce or be in connection with many cylinders.

After development the enamel becomes completely detached from its embryological substratum. The membrana tectoria, on the other hand, while becoming free in greater part, keeps its primitive connections with the crista spiralis, the least active part of its anatomical substratum.

These results concerning the genesis of the tectorial membrane are of general biological interest, for they prove the importance of the intercellular substance, the terminal bars, in the origin of special constituents of organs considered as of cuticular nature, now established for the membrana reticulares of sensorial organs. In this respect the intimate structure, fibrillar or reticular, of the prisms or of some segments of the membrana tectoria must be regarded as of secondary interest.

The present investigation has been carried out in the anatomical laboratory of the medical school of the Western Reserve University, Cleveland, Ohio. It is an agreeable duty to me to express my very sincere thanks to this university and to the staff of the anatomical department, in which I received gracious hospitality for nearly two years and was allowed to continue my scientific researches. My best thanks go mainly out to Professor Dr. T. Wingate Todd, who put at my disposal all the material, the reagents, and the instruments needed for my investigation. Owing to his kindness, to his ability and assistance, I was able to perform this work. I am very happy to pay to him the tribute of my deepest and most heartfelt gratitude. I also thank very much Mr. G. G. Marshall, who supplied me with a very interesting collection of bats.

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EXPLANATION OF FIGURES.

[All figures are photo micrographs taken at a magnification of 750 diameters, except figure 3, which is 400 diameters, and figure 23', which is 800 diameters. By the reproduction they have been reduced one-tenth.]

ABBREVIATIONS.

ar.,	areolar tissue.	mtcr.,	membrana tectoria formed on the surface of the crista spiralis.
bm.,	basement membrane.	mtg.,	membrana tectoria formed on the surface of the greater ridge.
cp.,	central corpuscle.	mtl.,	membrana tectoria formed on the surface of the lesser ridge.
cy.,	cylinders or prisms of membrana tectoria cut lengthwise.	n.,	nuclei.
cy',	cylinders or prisms of membrana tectoria cut transversely.	nb.,	nuclear bands of epithelial cells.
cy'',	cylinders or prisms cut tangentially.	nd', nd'', nd''',	nuclei of the three rows of cells of Deiters.
d', d'', d''', d''',	apices of the four rows of Deiters cells.	ner.,	nerve-fibers.
ep.,	epithelial cells.	ni.,	nuclei of the inner hair-cells.
essp.,	epithelial cells of the future sulcus spiralis.	nip.,	nuclei of the inner pillars.
f.,	clear cytoplasmic fields.	nisu.,	nuclei of the inner supporting cells.
f',	dark cytoplasmic fields.	noh', noh'', noh''',	nuclei of the three rows of outer hair-cells.
f'',	filamentous fields.	nop.,	nuclei of the outer pillars.
fb.,	fibrillar bundles or superficial segments of the bodies of outer pillars.	ns.,	nuclei of the sensorial cells.
gr.,	greater epithelial ridge.	nsu.,	nuclei of the supporting cells.
hs.,	superficial horseshoe-like elements, the bases of the acoustic hairs.	oh.,	outer hair-cells.
ic.,	intraepithelial connective-tissue.	oh', oh'', oh''', oh''',	apices of the four rows of outer hair-cells.
ih.,	apices of the inner hair-cells.	op.,	apices of the outer pillars.
ip.,	apices of the inner pillars.	pb.,	protoplasmic bands of epithelial cells.
is.,	apices of the inner supporting cells.	per.,	periosteal membrane.
l.,	longitudinal lines of the recently formed parts of membrana tectoria.	pr.,	prolongations of connective-tissue cells.
l',	transverse lines of the recently formed parts of membrana tectoria.	s', s'', s''',	three different successive planes of recently formed part of the membrana tectoria at surface of the lesser epithelial ridge.
lr.,	lesser epithelial ridge.	sf.,	sensorial fields.
m.,	superficial mosaic.	sph.,	attraction sphere.
mc.,	superficial mosaic of crista spiralis.	ssp.,	future sulcus spiralis.
mg.,	superficial mosaic of greater epithelial ridge.	st.,	subepithelial part of teeth of Huschke.
mh.,	superficial mosaic of Hensen cells.	suf.,	fields of the supporting cells.
mi.,	mitotic figures.	t.,	teeth of Huschke.
min.,	superficial mosaic of axial segment of the greater ridge.	tb.,	terminal bars.
ml.,	superficial mosaic of lesser ridge.	tb',	dense part of the membrana tectoria.
ms.,	striated membrane or apices of inner pillars.	tb'',	terminal bars alternately thicker and thinner.
mt.,	membrana tectoria.	zd.,	zona dentata of the crista spiralis.
mt',	recently formed part of membrana tectoria.	zp.,	zona papillaris of the crista spiralis.

DESCRIPTION OF PLATES.

PLATE 1.

1. Photograph of transverse section through tympanic wall of cochlea near apex. New-born dog. Fixation, trichloroacetic acid; stain, iron hematoxylin, Congo red.
2. Photograph of transverse section through second turn of spiral duct. Pig embryo 93.5 mm. Trichloroacetic acid; iron hematoxylin, Congo red.
3. Photograph of transverse, slightly oblique section through the greater and lesser ridges of second turn of cochlea. New-born dog. Trichloroacetic acid; iron hematoxylin, Congo red.
4. Photograph of section tangential to surface of the two epithelial ridges in second turn of cochlea. New-born dog. Trichloroacetic acid; iron hematoxylin, Congo red.
5. Photograph of section tangential to surface of the two epithelial ridges between second and third turn of cochlea. New-born dog. Trichloroacetic acid; iron hematoxylin, Congo red.
6. Photograph of section tangential to surface of crista spiralis and the greater epithelial ridge. Pig embryo 137 mm. Bouin's fluid; iron hematoxylin, Congo red.
- 7, 7'. Photographs of a section tangential to the surface of the organ of Corti in the adult bat (*Vespertilio fuscus*). Bouin's fluid; iron hematoxylin, Congo red.
8. Photograph of section tangential to surface of crista acustica. New-born dog. Trichloroacetic acid; iron hematoxylin, Congo red.
9. Photograph of section tangential to surface of crista acustica in adult bat (*Vespertilio fuscus*). Zenker's fluid; iron hematoxylin, Congo red.
10. Photograph of section tangential to the surface of crista acustica in adult white rat. Trichloroacetic acid; iron hematoxylin, Congo red.

PLATE 2.

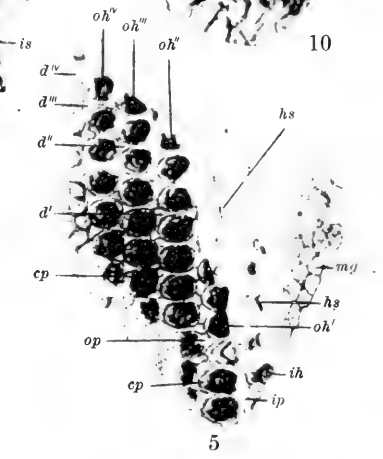
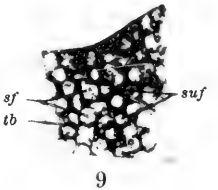
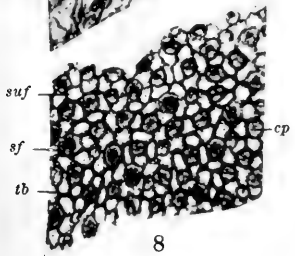
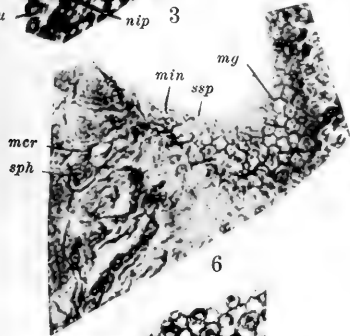
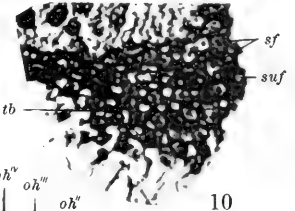
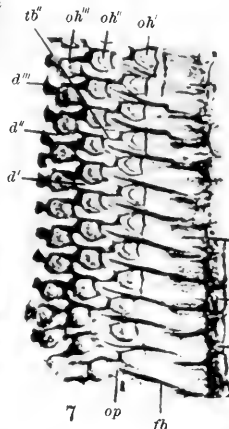
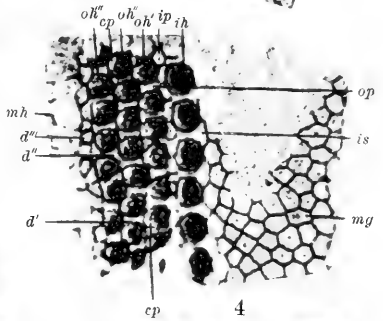
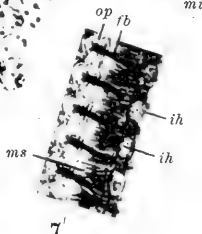
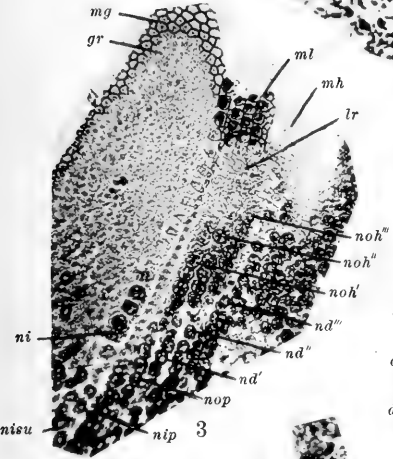
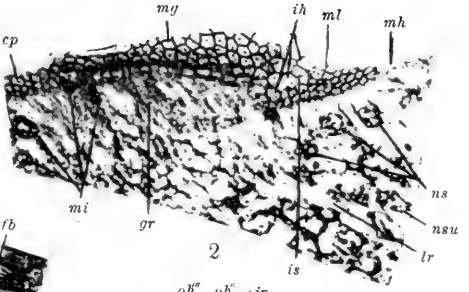
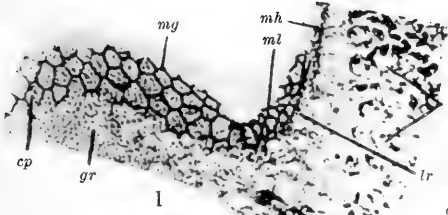
11. Photograph of section tangential to surface of macula acustica. Adult mouse. Trichloroacetic acid; iron hematoxylin, Congo red.
12. Photograph of section vertical to surface of the crista spiralis of second turn of cochlea. Pig embryo 127 mm. Bouin's fluid; Mallory's stain.
13. Photograph of section tangential to the surface of the crista spiralis of the third turn. Pig embryo 95 mm. Zenker's fluid; iron hematoxylin, Congo red, light green.
14. Photograph of section tangential and slightly oblique to surface of crista spiralis. Pig embryo 127 mm. Trichloroacetic acid; Mallory's stain.
15. Photograph of section tangential to surface of crista spiralis. Pig embryo 137 mm. Bouin's fluid; Mallory's stain.
16. Photograph of section vertical to surface of crista spiralis. Pig embryo 127 mm. Trichloroacetic acid; Mallory's stain.
- 17, 18. Photographs of section tangential to surface of crista spiralis of the third turn. New-born dog. Trichloroacetic acid; iron hematoxylin, Congo red.
19. Photograph of section tangential to the crista spiralis. Pig embryo 127 mm. Trichloroacetic acid; iron hematoxylin, Congo red.

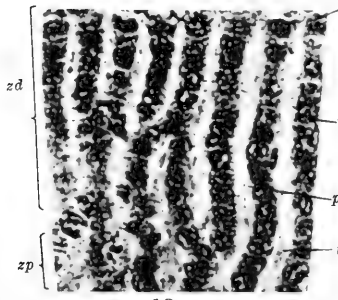
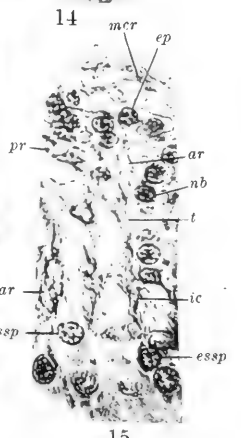
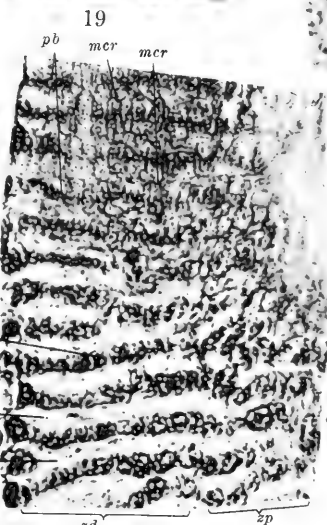
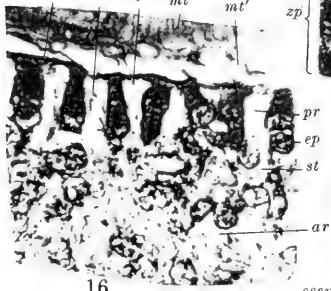
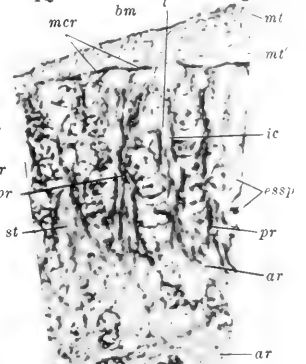
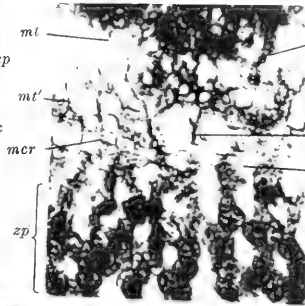
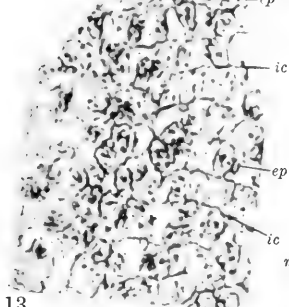
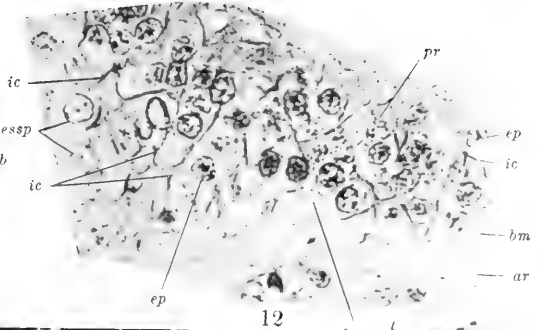
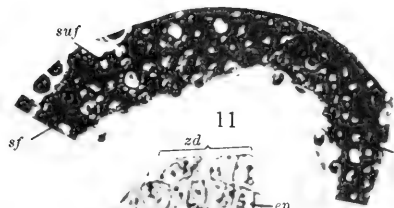
PLATE 3.

20. Photograph of section vertical to surface of crista spiralis. Pig embryo 190 mm. Bouin's fluid; Mallory's stain.
21. Photograph of section vertical to surface of crista spiralis. Young dog, age 4 months. Trichloroacetic acid; iron hematoxylin, Congo red, light green.
22. Photograph of section tangential to surface of crista spiralis. Pig embryo 190 mm. Trichloroacetic acid; iron hematoxylin, Congo red.
23. Photograph of section tangential to surface of crista spiralis in adult bat (*Vespertilio fuscus*). Zenker's fluid, iron hematoxylin, Congo red.
24. Photograph of section tangential to surface of crista spiralis. Young dog. Bouin's fluid; iron hematoxylin, Congo red.
- 25, 25', 26, 26'. Photographs of sections tangential to surface of greater ridge. New-born dog. Trichloroacetic acid; iron hematoxylin, Congo red.
27. Photograph of section tangential to surface of greater ridge. Pig embryo 93.5 mm. Fixation by uranium nitrate method of Ramon y Cajal.

PLATE 4.

28. Photograph of section tangential to surface of the two epithelial ridges and the crista spiralis and their membrana tectoria, between the second and third turns of cochlear duct. New-born dog. Trichloroacetic acid; iron hematoxylin, Congo red.
- 29, 30. Photographs of sections vertical to surface of the greater epithelial ridge. Pig embryo 95 mm. Zenker's fluid; iron hematoxylin, Congo red, light green.
31. Photograph of section vertical to the surface of the greater epithelial ridge. Pig embryo 95 mm. Zenker's fluid; Mallory's stain.
32. Photograph of section tangential to surface of the greater epithelial ridge. Pig embryo 127 mm. Trichloroacetic acid; iron hematoxylin, Congo red.
33. Photograph of section tangential to surface of organ of Corti and its superficial membrana tectoria. Pig embryo 150 mm. Zenker's fluid; iron hematoxylin, Congo red, light green.
34. Photograph of section tangential to surface of crista spiralis. Pig embryo 127 mm. Trichloroacetic acid; iron hematoxylin, Congo red.
35. Photograph of section tangential to surface of membrana tectoria. Adult rat (*Mus decumanus*). Bouin's fluid; iron hematoxylin, Congo red, light green.
36. Photograph of section tangential to surface of membrana tectoria. Pig embryo 150 mm. Bouin's fluid; Mallory's stain.





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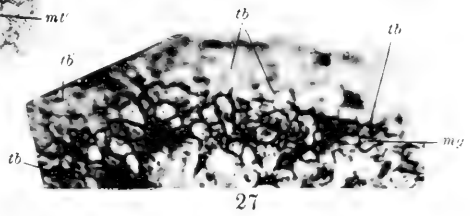
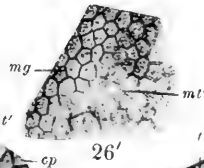
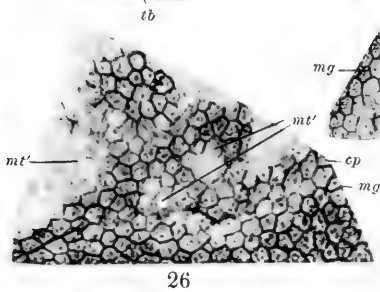
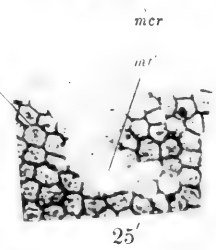
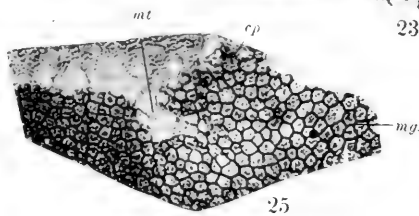
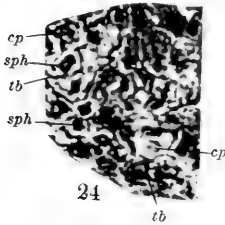
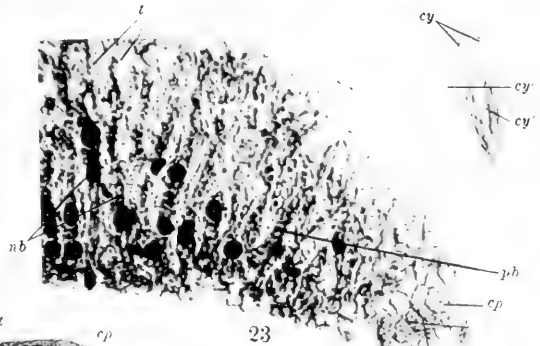
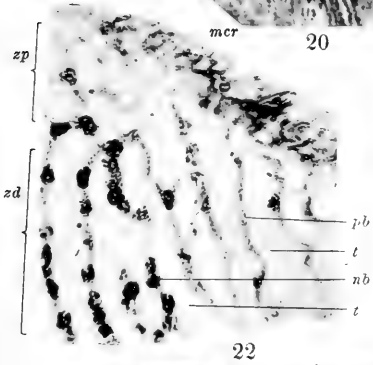
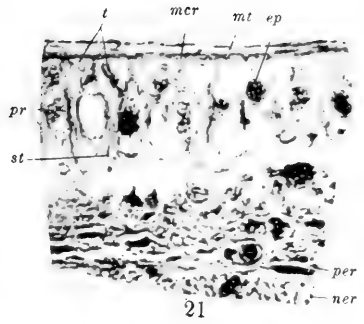
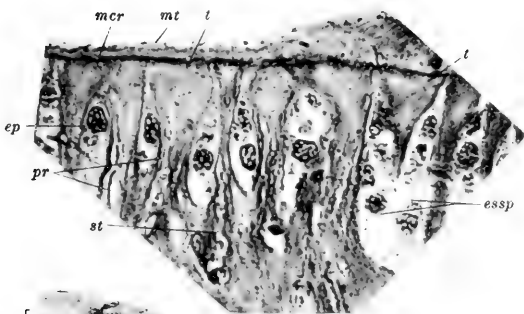
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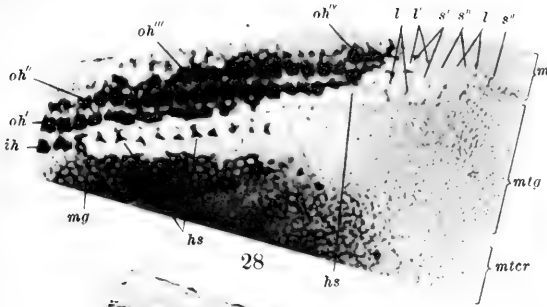
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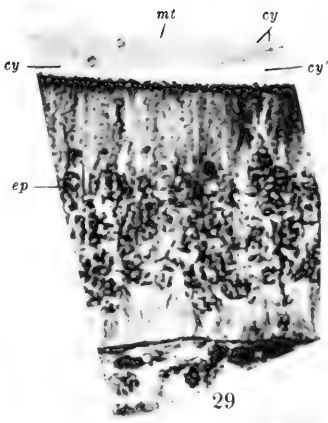
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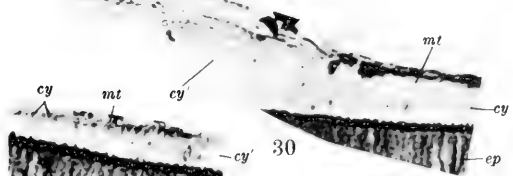




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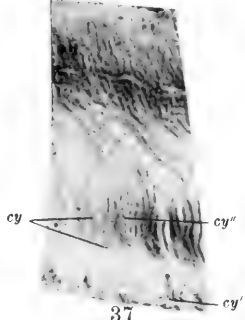
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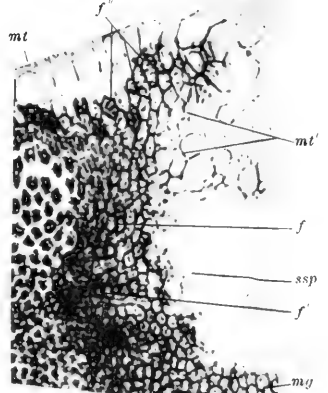
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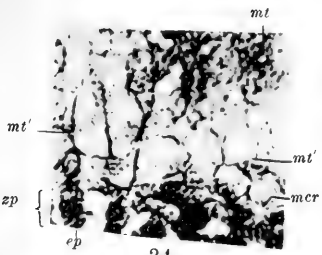
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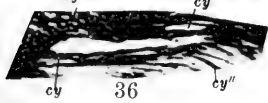
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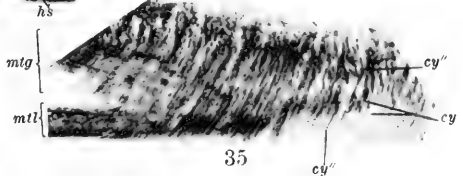
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CONTRIBUTIONS TO EMBRYOLOGY, No. 22

STUDY OF A HUMAN SPINA BIFIDA MONSTER WITH
ENCEPHALOCELES AND OTHER ABNORMALITIES.

BY THEODORA WHEELER.

With four plates.

STUDY OF HUMAN SPINA BIFIDA MONSTER WITH ENCEPHALOCELES AND OTHER ABNORMALITIES.

BY THEODORA WHEELER.

The specimen described in this study is a human female monster with spina bifida, in which there is total subcutaneous involvement of the spine and a defective occiput. The thoracic and cervical regions of the spine are much shortened, and encephaloceles and numerous other abnormalities are present. The type, though a rather unusual variety of spina bifida, occurs frequently enough to have been recognized and grouped by itself for some time past, and to this group the term *iniencephaly* has been applied. Because of the striking appearance of these specimens, one or more are usually to be found in any museum of pathology. In the embryological collection of over 1,600 specimens belonging to the Department of Embryology of the Carnegie Institution of Washington, the only example is the one presented in this paper, No. 862*a*. It was through the courtesy of the Bridgeport General Hospital that this specimen was obtained.

Only a short review of the literature on spina bifida will be given here. More complete historical accounts with extensive bibliographies are to be found in articles by Kermauner and by Ernst in Schwalbe's *Morphologie der Missbildung* (1909), and also in a chapter on spina bifida by Tillmann, in his volume of *Deutsche Chirurgie*, v. 62*a* (1905). The earliest references to many teratological conditions are supposed to be found in folklore and in mythological tales of centaurs, cyclops, mermaids, and such creatures, and it has been suggested that among such stories may likewise be found the first record of spina bifida. Possibly the hairy and cloven-hoofed satyr was originally a fairly normal individual with spina bifida, hypertrichosis, and club feet, whose abnormalities gradually developed through excited hearsay into the hind-quarters of a beast. Even in recent times, in connection with scientific work on this condition, such inaccuracies have only too frequently been paralleled by superficial observations and indefinite speculations. However, it is not surprising that a good deal of vagueness has existed in regard to spina bifida, as the subject-matter includes widely dissimilar and very complicated conditions.

As described among human forms, two chief types are distinguished: the flat-spine type (*rachischisis*, *spina bifida aperta*) and the subcutaneous type (*cystic, occulta*). In both of these forms the greatest variations exist as to location and amount of spinal involvement. In some instances only a single segment is affected; in others the whole spinal column, together with the cranium, may be involved. Combinations of these two forms are to be found, and also conditions where the two varieties merge into one another. Associated with every type of the condition are found innumerable other abnormalities.

Owing to this mass of complicated material and to the widely different nomenclatures used by the large number of investigators who have worked on the problem, the literature is enormous and rather confused. The classification is still very superficial. In teratology, as in general pathology, the trend has been to supplant classifications based on regional distribution by those having an etiological basis. There exists still in the literature on spina bifida a great deal of the former method. This is due to the fact that until quite recently study has been of external form alone, from which method only a crude regional classification can result. By the application of the more penetrating methods of modern anatomy, embryology, and experimental biology, progress has been made toward etiological classification.

In 1881 Koch assembled a number of different forms of spina bifida. He pointed out the distinction between the flat-spine form, in which the spinal cord is uncovered (spina bifida aperta), and the cystic or subcutaneous form, in which the soft parts have joined but the bony arches remain ununited. He attributed a later formative period to the subcutaneous than to the open form. In 1886 von Recklinhausen presented over 30 specimens of spina bifida and focussed attention especially upon the pathological anatomy of the central nervous system and its membranes in the fetal and older forms. By thoroughly analyzing the conditions met with and applying the conception of arrested development, he was able to offer reasonable interpretations for much of the developmental mechanism which up to that time had not been understood. Contemporaneously with these two writers, and since their time, many aspects of the subject have been studied. The surgical treatment of spina bifida has been taken up by many, notably Bayer, Hildebrand, and Muscatello. Other authors have described special types of the abnormality. Among these may be mentioned Lewis's paper on iniencephaly. He collected 23 cases similar to the one herein described, which show some of the variations presented by this special form. In the literature are to be found fairly numerous descriptions of young specimens with spina bifida. In "A study of the causes underlying the origin of human monsters" (1908), Mall describes 12 from his collection and cites several others from the literature. An interesting 8 mm. ferret embryo with localized cervical hydromyelia was described by Good in 1912.

Experimental studies on the lower animals have formed a very important source of information with regard to the open variety of spina bifida. In Mall's paper a review of the literature on the subject up to 1908 is given. The work of Hertwig and Morgan has attracted especial attention, the former showing that external agents causing delay in the closure of the blastopore can bring about embryological spina bifida. The work of the latter author has been interpreted as pointing toward NaCl as the definite etiological agent, as he was able to produce a delayed closure of the blastopore in frogs' eggs through the use of a 0.6 per cent solution of NaCl. Embryological spina bifida has also been produced occasionally in chicks by overheating and various other methods. Working with frogs' eggs, by ultra-violet-ray exposures Baldwin (1915) obtained a condition of doubled and closed neural canal and sometimes doubled cord. His specimens were usually

two-tailed. He referred to them as spina bifida and gave a clear explanation of the mechanics of the process producing them. However, the relation of this type of spina bifida to the more ordinary condition of a single open neural canal is not altogether plain, and his suggestion that "imperfect oxidation" causes spina bifida does not further clarify the question.

The earliest picture of the subcutaneous type of spina bifida with which we are familiar is that encountered in embryos around the 10 mm. stage of development, in which the neural tube is everywhere closed, showing, however, a greater or less area of enlargement. Such a state has not as yet been experimentally produced. Several explanations have been advanced to account for it, none of which are satisfying, nor substantiated by evidence. One suggestion is that the enlargement of the neural tube is due to the fact that dorsally it remains attached to the ectoderm (non-separation of the membrana reuniens). On the other hand, it has been suggested that the neural tube becomes enlarged because of increased pressure from the contained fluid. In this connection it would seem that when the affected areas are limited in extent they are in some way connected with the curvatures of the body, since such areas usually occur in the neck or sacral region, where, in the embryo, the curves are most pronounced. The process is supposed to be one of subsequent pressure of the hydromyelia on the surrounding parts, thus inhibiting the development of cartilage and bone. With our advance in knowledge regarding the circulation of the cerebro-spinal fluid, some of the most puzzling features presented by subcutaneous spina bifida will probably be satisfactorily explained. The work of Weed on the normal cerebro-spinal fluid circulation is most helpful, supplying as it does for the first time an adequately correlated picture of the formation and extension of the cerebro-spinal fluid with the differentiation of the perimedullary mesenchyme to form the meninges. In the meantime, any discussion concerning the etiology of subcutaneous spina bifida is entirely theoretical. Suggestions have been made that it may arise directly from the open spina bifida form; again, that it may be the result of some entirely different process, or that both forms may be produced by the same pathological agent acting at a different stage of development. While it has been generally assumed that the open and subcutaneous forms of spina bifida are related, this has never been proved. A more definite picture of each process must be obtained before we can know the nature of the relation, or whether there is such a relation, existing between the two. The literature on the subject gives the impression that, although sound facts and more or less sound theories regarding spina bifida have multiplied, there is much that is not clear and that must be understood before we can have a comprehensive insight into the processes producing it. That this information may be gained through a closer embryological study seems probable.

The study of the specimen dealt with in this paper has been made chiefly along morphological lines. Only a meager clinical history regarding it was obtainable; the child was illegitimate, was born spontaneously at full term, and lived only a few hours. Its external form is shown in figures 1, 2, and 3, and various measurements are given in table 2. Externally, the most marked abnormality is the

extreme dorsal flexion and shortening of the trunk. The head is drawn back close to the sacral region. The chest and abdomen are unusually prominent. The arms and legs are symmetrical and well developed, but the shoulders are hunched up and lie far forward, close to the cheeks. The face is directed upward, which throws the top of the head back so that the vertex lies level with the raised shoulders. The neck is obliterated and the chin and chest lie in one plane. The features are well formed with the exception of lack of prominence of the chin and deformity of the ears. Figures 1 and 8 show the right ear. The deformity of the left ear is similar. The anthelix is pushed outward so as to be unusually prominent; the tragus is shifted medially and upward, so that it lies opposite the concha; the antitragus lies below it, pressed against the cheek. Darwin's tubercle is present. The external auditory meatus is patent and the parts of the middle and inner ear prove on dissection to be well developed. The whole external ear is considerably narrowed, as is indicated by as low a physiognomical index as 48.5. The average physiognomical index of the right ear of 14 white infants under 3 weeks of age, in the obstetrical ward of the Johns Hopkins Hospital, was found to be 69.1, varying between 62.5 and 78.7. Measurements made by Dr. A. H. Schultz of 4 dead white infants not older than 1 month showed the physiognomical index of the right ear to be 65.0, with a variation between 60.0 and 73.1. Though the physiognomical index shows a rather wide variation due to the great flexibility of the ear cartilage in infants, nowhere in the small group of available normal cases is it nearly so low as in his specimen. The ear deformity is apparently caused by pressure upon and twisting of the external parts of the ear during their development by the backward-bent head and the shoulders which lie close on either side. Marx describes a deformed ear which he designates as "Wildermüthsche Ohr," in which the anthelix is very prominent. From the base of each ear a crease in the skin extends for 3 cm. medially under the chin to within 2 cm. of the midline, as is seen in figure 2.

INTEGUMENT.

The dome of the head is narrowed and flattened, and is covered with light-brown hair, 1 cm. in length. Just above and behind the ears the hair is 2 cm. long and is quite thick. Across the middle of the forehead at the hair margin is a narrow raised ridge of puckered skin, 2 cm. long. A section through this area shows the structures of the skin to be well developed and similar to the adjoining normal skin, except that where the surface is raised the papillæ are somewhat flattened underneath. The ridge is probably the result of rough handling before fixation.

From the back of the head protrude three encephalocetes in a horizontal line, extending from a point 6 cm. behind the tip of the left ear to a point 2 cm. behind the tip of the right ear. These are best displayed in figures 1 and 3. They measure 13 cm. horizontally along their superior margin, and the vertical diameter is 4.3 cm. The midline of the specimen comes between the left and middle encephalocetes. In the midline their vertical diameter extends from a point 3.6 cm. below the vertex to within 6 cm. of the anus. The middle swelling is the largest of the

three and is cone-shaped, whereas the other two are smaller and hemispherical. All are of soft consistency. The large conelike swelling measures 6 cm. from the superior margin to its tip and 2.5 cm. from tip to inferior margin, its base being circular and measuring 4.3 cm. in diameter. This cone lies pendant over the back; the proximal half of its superior surface is covered with scalp and fine brown hair 2.5 cm. long. A strip of coarser hair of the same length follows the median margin of the swelling to its lowest point. The covering of the distal half of the superior surface and the entire inferior surface resembles smooth, fine-grained leather. No hairs are present, but it is dotted with minute pores which on microscopical examination are seen to be the mouths of sweat-ducts. The wall of the sac is 3 mm. thick, a section through which shows an extremely thin layer of epidermis lying immediately over a vascular connective tissue, containing the sweat-glands mentioned above, but no hair follicles. There are two oval naevi near the tip of the sac, which in the gross resemble scars. These lie in the same long axis directed laterally through the tip of the sac. The smaller of the two is 5 mm. to the left of the tip and measures 7 by 3 mm. The larger lies 10 mm. to the right of the tip and measures 25 by 17 mm. The color of these areas is lighter than the surrounding tissue and the surfaces are stiff, smooth, and slightly raised. In their neighborhood the thickness of the sac wall is increased to 9 mm. Histologically the epithelium is lacking here and a very vascular connective tissue forms the raised surface. The lining of the upper part of the sac is smooth fibrous tissue continuous with the dura of the main cerebro-spinal cavity. Near the tip, however, it is made up of shaggy strands of blood-vessels whose complicated, interweaving pattern is like the early capillary plexus of the dura, as described by Streeter (1915). This suggests that the irregular vascularization in this region may be due to arrested development of the vascular system. There is marked engorgement of these vessels and congestion in all the tissues. Part of this extreme engorgement was probably caused by birth trauma.

The left encephalocele measures 5 by 4 cm. and protrudes 1 cm. from the surface. Its upper half is covered with fine hairs and the lower half with normal-appearing skin. The wall is 1 to 2 cm. thick, composed chiefly of a layer of subcutaneous fat. On its left lower border there is a rounded bleb of porous, wrinkled skin 1 cm. in diameter, over which are scattered a few hairs 2 cm. long, and which contains around its depressed circumference a much thicker growth of similar hairs. A section through the wrinkled skin shows that it lies over a funnel-shaped canal, the wider mouth of which extends down through 1.5 cm. of subcutaneous fat to the subdural space, where the canal becomes narrow. This canal is filled with fluid and contains a few blood-vessels supported by loose connective-tissue septa. Its walls are formed of rather dense connective tissue.

The right encephalocele is 4 cm. in diameter at its base, and the surface, which is covered with hairy scalp, is but slightly raised above the adjoining structures. Its wall, consisting of epidermis, connective tissue, and fat, is but 3 mm. thick.

A summing up of the integument findings shows that both normal skin and scalp are found over the areas adjoining the encephaloceles and over parts of the

encephalocles as well. In addition to this, in the adjoining areas there are hypertrichosis and thickened subcutaneous fat, varying from 5 to 25 mm. in thickness. The wall of the large encephalocle varies from 3 to 9 mm. in thickness and is formed by angiomatous tissue covered with a thin layer of epidermis penetrated by sweat-glands. In two places near the tip of the sac nævi are formed by the vascular tissue extending to the surface. The walls of the small sacs vary from 3 to 20 mm. and are formed chiefly by subcutaneous fat covered with scalp. On the left a pore and canal pierce through to the subdural space.

Spietseka in 1894 collected the various forms of skin changes associated with spina bifida. Besides the varieties here found, he described pigment blotches and such a marked increase of fatty tissue as to amount to lipomata. In an article on skin anomalies by Bettmann, in Schwalbe's *Morphologie der Missbildungen*, nævi are noted as among the most frequent anomalies.

METHODS.

A sagittal section was made of the specimen under discussion (see fig. 9). The spinal column shows extreme lordosis, undeveloped arches throughout, and shortening and fusion of the upper vertebræ. The central nervous system is very much disturbed, a large part of it having slipped down below the cranium, through a much enlarged foramen magnum. This portion lies on the thoracic and lumbar vertebræ and protrudes into the sacs already described. The brain and cord were removed, and a clay impression was made of the entire space occupied by the central nervous system. This was then cast in wax and photographed, as shown in figures 4, 5, 6, and 7. By the help of this model the general shape taken by the central nervous system was demonstrated and the study of its internal arrangement and relation to other structures was facilitated. The consideration of these will be taken up later in this paper.

In the sagittal section, thick subcutaneous pads of fat are seen in the undifferentiated region of the neck between chin and thorax, above the symphysis, and over the sacral region. Dissection shows this subcutaneous fat to be likewise particularly abundant over the back and shoulders. There is also found an extreme grade of undeveloped or split soft palate, associated with which is a bilateral anlage of the uvula, that on the left side being shown in figure 9a. Consideration of the normal development of the soft palate will help to indicate how this defect originated. It is generally agreed that at a very early date the tongue occupies the area which is later occupied by the septum and palate. The normal rearrangement of these parts to their final positions is accomplished by medial growth of the palate and downward growth of the septum, associated with independent shifting of the tongue. If for any reason the tongue can not withdraw, the palate remains split to a greater or less degree. That such a cause was operative in this specimen seems likely; the distorted position of the cervical spine might easily have caused a crowding in the adjoining pharyngeal region and so prevented the tongue from receding.

SKELETON.

A dissection of the skeleton was made, the vertebræ and ribs being left connected by their ligaments, so that the specimen could be easily mounted. To facilitate handling, two transverse cuts in the skeleton were made at the level of the first thoracic and first lumbar vertebræ. A study of the skeleton shows marked maldevelopment and distortion, as may be seen in figures 10, 11, and 12. The axial skeleton is most affected, the arches of all the vertebræ being defective; these are open posteriorly in the midline and are flattened outward, forming wide anterior support for the central nervous system. In the cervical and thoracic regions the bodies of the vertebræ are fused, shortened, and dorsally flexed, so that the spine is bent almost double. The occiput actually rests on the gaping vertebral arches and fuses with them.

Viewing the occiput in figures 13 and 14, the inferior and medial two-thirds of the squama occipitalis is seen to be defective. A bilateral bony exerescence on its dorsal surface, near the defective medial margin of the squama and close to its junction with the partes laterales joins it to the everted arches of the second lumbar vertebra on the left side and to the first lumbar vertebra on the right. The defect of the squama in the midline, together with a widening of the angles formed by the junction of the pars basalis with the partes laterales, has greatly increased the size of the foramen magnum. This is oval in shape and measures 4.5 by 3.7 cm. The long diameter is antero-posterior, and posteriorly it slants slightly to the left. For purposes of comparison the size of a normal foramen is indicated in figure 13 by means of dotted lines. The large foramen resembles that of the chondrocranium at a very early stage of development. The participation of both the squama occipitalis and the vertebral arches in the midline defect, as it exists here, has been regarded as teratological evidence of the homology of these parts, and probably has been a factor in advancing the opinion, which has slowly gained ground, that some cranial defects, even when existing alone, belong in the same category with certain vertebral abnormalities.

The two partes laterales are well formed and but slightly asymmetrical. The left jugular process is more marked than the right. On the left inferior surface directly under the jugular process there is a cartilaginous prominence which meets the tip of the transverse process of the underlying atlas. The hypoglossal foramen on the left side is a single canal, and while the right hypoglossal foramen has a single perforation on the medial surface of the pars lateralis, it has a double exit on the outer surface of the bone. A small rod of bone divides it into a smaller anterior and a larger posterior foramen, as is demonstrated in figure 11. A division similar to this has been observed frequently in embryological studies and appears on the left side in a skull of a human fetus modeled by Macklin. The condition is of rather frequent occurrence. Lillie gives a ratio of 14 per cent complete division and 36 per cent indicated division, out of 305 left and right canals examined by him. The explanation generally offered is that it is persisting tissue from primitive cranial divisions which usually disappear at a very early stage.

In our specimen the pars basalis of the occiput is oval and asymmetrical along its inferior margin, as shown in figure 11, and measures 18 by 14 mm. Its sphenoidal margin is 6 mm. thick, and the thickness of the bone elsewhere is 3 mm. Its posterior surface is slightly concave, there being a rather deeper depression immediately under the sphenoidal articulation than elsewhere. The anterior surface of the pars basalis is nearly flat. The inferior margin has a notch near the midline and on either side of this the bone projects downward, 2 mm. on the right and 4 mm. on the left. A slit-like foramen 2.7 mm. wide directed forward and upward pierces the pars basalis near its center. On each temporal bone the eminentia arcuata is very prominent and the fossa subarcuata deeply depressed below. The ear ossicles are well developed and the other relations of the bone are normal. With the exception of the small size of the cranial vault the rest of the skull is well formed.

The bodies of all the cervical and thoracic vertebrae and the dorsal surface of the first lumbar vertebra are fused together in a bent and irregular central plate of cartilage containing irregular ossification centers. The roots of the arches and the ribs project from this plate. The relations of the various parts are shown in figures 10, 11, and 15. At the superior end of this plate the foveal surfaces of the atlas and its transverse processes are distinguishable, but, as may be seen in figure 10, both posterior and anterior arches are lost. The foveae are shifted to the right in relation to their transverse processes, as may be seen in figure 11. This shifting causes the right atlantal transverse process to lie immediately under the fovea. The left is uncovered by the fovea on that side, but is fused at its tip with the left pars lateralis.

Viewed from the side in figure 12, the cervical and lower thoracic portions of the central vertebral plate form the two arms of a wide-mouthed U, while the bent base of the U occurs in the plate from the level of the first to the sixth ribs. Besides this marked lordosis, there is a very slight lateral bend which shows in the dorsal view of the skeleton (fig. 10), giving the vertebral plate a slightly curved S-shape. (This condition of scoliosis and lordosis in varying degrees is very frequently noted in the extreme forms of spina bifida.) The concavity at the right margin of the vertebral plate is opposite the first rib and at the left opposite the sixth rib. From the central plate of this specimen throughout its extent the radices project outward on both sides and formed between them are two uneven rows of intervertebral foramina. The processes are tiny spicules of bone in the cervical and upper thoracic region, becoming larger in the lower part of the column. In the cervical region 7 radices are distinct and 12 in the thoracic region. The former could not all be shown in the drawings.

The arches of both cervical and thoracic regions are everted and fused. This formation, together with the antero-posterior bend of the plate, makes a rather deep pocket of bone which contains parts of the much disturbed central nervous system. The lumbar and sacral vertebral column is much less affected than the upper part. The dorsal part of the first lumbar vertebral body is fused with the thoracic vertebrae, its ventral surface, however, being distinct. The four lower

lumbar and the five sacral vertebral bodies are well formed, as are the transverse processes of all the lumbar vertebræ and the partes laterales of the sacrum (figs. 10 and 12). The first four lumbar arches are everted, as are the thoracic arches, though individually they are distinct and not fused. The fifth lumbar and the five sacral arches are incomplete, but project medially toward one another and are not everted. The lumbar column is 4.3 cm. long and the sacrum 3 cm. in length. The coccyx is composed of four segments, which measure 1.6 cm. and are bent to the left. In studying the proportions of the vertebral column, Acby's tables of relations in normal vertebral columns in the new-born were used, with the results shown in table 1.

TABLE 1.—Comparison in millimeters of the vertebral lengths of specimen with those given by Acby for normal new-born.

	Total.	Cervical.	Thoracic.	Lumbar.
Acby (normal)	176.5	45.1	83.9	47.5
No. 862 a	105.0	15.0	45.0	43.0

A comparison of these vertebral lengths shows the lumbar portion of this specimen to be within the limits of normal, though near the minimal margin. The cervical portion is less than half, and the thoracic portion a trifle more than half the length of the normal. Acby gives 26.4 mm. for the transverse diameter of the atlas, 12.2 mm. for the width of the body of the sixth thoracic vertebra, and 17.5 mm. for that of the fifth lumbar. In this specimen the lateral limits of the foveæ are 31.0 mm. and the width of the transverse processes of the atlas 38.0 mm. The width of the vertebral plate in the midthoracic region is 23 mm. and the width of the fifth lumbar vertebra is 21 mm. These differences show an irregular widening process to have taken place in the vertebral bodies themselves, the change being most marked in the thoracic and cervical regions. The absence of lateral pressure from ununited arches must have been an important factor in this broadening process.

There are twelve ribs on each side which have undergone considerable disturbance. On the right, the first six are fused near their bases (figs. 10, 11, and 15). The second rib terminates at the end of its proximal third in a plate of bone by which it is joined to the first and third ribs. On the left, the fifth to ninth ribs are crowded together in their proximal half (figs. 10, 12, and 15). The fifth and sixth have but one costal cartilage between them. The sixth and seventh ribs are fused for a few millimeters just proximal to their termination. Further fusion occurs in pairs at the bases of the following ribs: on the right, between seventh and eighth, ninth and tenth; on the left, between the first and second, third and fourth. This shows on the ventral surface in figure 15.

The sternum, as seen in figure 16, has four ossification centers near the median line at the level of the first costal cartilage and of the first, second, and third left intercostal spaces. There are six costal cartilage connections on each side. The last on each side, however, belongs to the seventh rib. The discrepancy occurs on the right side through the aborted second rib and on the left side through the

fifth and sixth, having but one cartilage between them. The first and seventh costal cartilages of the two sides are opposite each other. The arrangement of the other cartilages is such that the third to the fifth on the left side are from 0.5 to 1 cm. lower than the corresponding cartilages on the right, yet not quite opposite the succeeding one. A small cartilaginous knob (2 by 5 by 3 mm.) above the manubrium is a persistent episternum. The measurements of the sternum are given in table 2.

TABLE 2.—*Dimensions in centimeters.*

	<i>cm.</i>		<i>cm.</i>
Body lengths:		Trunk— <i>Continued.</i>	
Vertex-anus	14.0	Diameter of right nipple	0.9
umbilicus	32.0	left nipple7
Lower hair border-anus (length of back)	10.0	Sternum, episternum, and zyphoid:	
Head:		Length of sternum with episternum and	6.9
Circumference of head	32.5	zyphoid sternum alone	6.0
Biparietal diameter	8.5	Width of sternum	1.0
Anterior fontanel	2.7 cm. transverse by 2.5	Thickness of sternum3
Posterior fontanel	1.6 cm. " by 1.7	Length of episternum3
Face, vertical length (border of hair to chin) ..	9.5	Length of zyphoid	9.6
Clear breadth (from free edges of tragi)	9.2	Extremities:	
Eyes apart	1.8	Upper arm (circumference of both left and	
Nose across	2.3	right)	9.0
Mouth across	2.0	Lower arm (circumference of both left and	
Trunk:		right)	7.0
Circumference at umbilicus, passing around the		Hand with middle finger	6.0
back at base of middle sac	33.0	Right trochanter—heel	20.9
Distance across shoulders	12.0	Right foot	7.5
Nipples apart	6.4		

The two scapulæ which are shown in figures 18 and 20 are distorted, as will be seen by comparison with figures 17 and 19, representing normal left and right scapulæ. In both the pathological bones the supraspinous portions are poorly formed and the inferior vertebral margins are concave. Graves designates a concavity of the vertebral margin of the scapula as scapula scaphoidea. He notes that it is of fairly frequent occurrence and claims that it is associated with syphilis in the parents. He gives as his figures, however, no definite rate of occurrence. Here it may be mentioned that the Levaditi stain done on the tissues of this specimen showed no spirochætes. On the right scapula the vertebral margin passes as a straight line from the medial termination of the spinous process to the incisura next the glenoid process. The vertical diameter of the right scapula measures 36 mm. from the tip of the cartilaginous process at the inferior angle to the superior margin near the incisura. Its transverse diameter along the base of the spinous process, near the termination of the latter, to the center of the glenoid fossa is 26 mm. The subscapular angle is 128°, the infraspinous angle is 122°, and the supraspinous angle is 110°. On the left scapula, the vertebral margin above the spinous process projects at a fairly sharp angle near its middle. The vertical diameter taken from the tip of the inferior angle to the end of the projecting point of the supraspinata is 29 mm. The horizontal diameter of the left scapula, measured similarly as the right, is 36.5 mm. The vertebral margin of the left scapula at the termination of the spinous process is elongated by a bony and cartilaginous knob, which is attached to a curved rod of bone 10 mm.

long and 2 mm. in diameter. This rod is joined at its other end to the everted arches of the vertebræ underlying it. On the left scapula the subscapular angle is 117° , the supraspinous angle is 109.5° and the infraspinous angle is 133.5° . The left scapula shows a rather interesting condition, presenting three out of four features often associated with Sprengel's deformity (congenital elevation of the shoulders). These are, according to Horwitz: (1) changed relations of the diameters to each other; (2) bending forward of the supraspinous process; (3) prolongation or rounding of superior median angle; (4) presence of exostoses and articulations with the vertebral column.

In this case the exception to the above conditions is the superior median angle, which can hardly be called prolonged. Scapular measurements of the new-born could not be found in the literature, but two supposedly normal sets were obtained from mounted skeletons belonging to the Obstetrical Department of the Johns Hopkins Hospital, and the measurements of several other scapulæ were available through the courtesy of Dr. A. H. Schultz.

TABLE 3.—Comparison in millimeters of the scapular measurements of specimen with those of several normal new-born.

Specimen.	Vertebral length.	Transverse diameter.	Vertical diameter.	Morphological index.
Schultz, No. 3.....	165	R. 24 L. 24	R. 33 L. 31	R. 72.7 L. 77.4
Schultz, No. 4.....	151	R. 29 L. 30	R. 38 L. 38	R. 76.3 L. 78.7
Obstetrical Department skeleton..	169	R. 27 L. 27	R. 36 L. 35	R. 75.0 L. 77.1
Do.....	172	R. 26 L. 26	R. 36 L. 29	R. 72.2 L. 106.8
No. 862 <i>a</i>		L. 31		

Table 3 shows that the ratio of the diameters of the right scapula of 862*a* is near those of the supposedly normal bones. The left scapula, on the other hand, has the relations of its diameters reversed. The transverse diameter exceeds the vertical. Thus its morphological index is 106.8, while none of the normal indices exceeds 80. The subscapular angle on the left side is somewhat smaller than on the right. The bony articulation joining the left scapula to the vertebral column is attached in the upper third of the vertebral scapular margin as in most of the Sprengel deformity cases. Some interest is attached to this abnormal bony spicule and various suggestions have been made concerning it. The opinion seems to be generally accepted that it arises from its scapular end. Cases are recorded in which other anomalous bones are joined only to the scapula, and their occurrence substantiates this view. Case XVI in von Recklinhausen's paper is a monster very like No. 862*a*. In it there is "ein 1 cm. langer knöcherner, rippenartiger, am oberen Winkel des knorpeligen Schulterblatts articulirender Körper." Gruber gives a case found in an adult male cadaver of a "fortsatzartigen, cylindrischen Höcker an der Vorderfläche des Angulus superior der Scapula." No satisfactory hypothesis has been advanced to further explain the origin of these bones. The length of the right clavicle is 39 mm. while the left clavicle measures 34 mm. and is slightly

more bent at its distal end than the right. This shortening of the clavicle on the side of the abnormal scapula is frequent in Sprengel's deformity. The hunched position of the shoulders, so prominent externally in this case, may be seen to be due to the defective cervical and upper thoracic vertebræ, which lie crumpled to half their normal length under the scapulæ, their normal relations to these bones being quite changed.

MUSCLES.

The region of abnormal musculature corresponds, as would be supposed, to the skeleton derangements. This is limited to the neighborhood of the axial skeleton, where the affected muscles are both under and intermingled with an unusually large amount of fascia. On superficial dissection, the topmost layer of muscles is well formed, except for the trapezius, which is represented similarly on the two sides by thin strap-like bands of muscle, 3 by 1 cm. The fibers run parallel with the long diameter, from the origin of the muscle, situated in fascia lying over the everted and crumpled cervical and thoracic vertebral arches, to their insertion on the acromial extremity of the clavicle, the acromion, and spine of the scapula. Those fibers which insert on the scapular spine have become folded under the others, owing to the contracted and lowered origin of all the fibers. A condition of the trapezius similar to this has been noted in a case of total rachischisis given by Kermauner, in which case, also, lordosis and marked shortening of the spine were the underlying skeletal conditions. As Kermauner says, the association of this variety of muscle and bone defect is only natural, "for, with the marked shortening of the trunk, there necessarily exists a reduction in the cranio-caudal diameter of the muscles of this region."

Upon further dissection, the condition of the underlying muscles was determined. The rhomboidei are represented bilaterally by very thin and short muscles, only 3 mm. in length. They arise from the connective tissue over the fused and everted arches of the thoracic vertebræ, and are inserted in fascia along the inferior vertebral borders of the scapulæ. (In Le Double's work a reduction in the thickness of these muscles is recorded.) The two levator scapulæ are present. They arise from the fused transverse processes of the upper cervical vertebræ and are inserted in fascia along the superior vertebral margins of the scapulæ. There is no reduction in the size of either muscle. They are directed horizontally out instead of slanting downwards as usual. This is due to the scapulæ lying directly over the cervical vertebræ. A cross-section of the left muscle at its origin is shown in figure 21. Figure 18 shows the left scapula and the rhomboideus and levator scapulæ muscles inserted in fascia which forms a sheet between the irregular projections of the vertebral margins of the bone. The abnormal spicule of bone is attached to the scapula at the median angle between the insertions of the levator scapulæ and the rhomboideus.

On each side most of the dorsal muscles consist of an irregular longitudinal bundle which extends along the sides of the vertebræ from sacrum to occiput and which sends scattered projections on to the ribs. Under this bundle in the lumbar region the quadratus lumborum and psoas muscles lie undisturbed. On the left

side this bundle is shown somewhat diagrammatically in figure 21 and labeled *sacrospinalis*. In the lumbar region it is cylindrical and measures 1.5 cm. in diameter. It grows flatter and broader as it nears the upper part of the spine, this formation being due to a state of arrested development of the sacrospinalis and short back-muscles. The early condition of dorsal musculature which it simulates is strikingly illustrated in Bardeen and Lewis's model of an 11 mm. embryo (1901), where a bundle distinct from the ventral-lateral muscles lies bilaterally in the trough formed at the sides of the vertebrae. In another model given in the same paper of a 20 mm. embryo, the bundle may still be seen lying under the connective tissue of the region, and this divided condition of the back-muscles persists normally until about the 60 mm. stage.

The *serati posterior inferior* are shown by projections from the dorsal bundle which on both sides cover the proximal half of the three lowest ribs. In the upper thoracic region, lying on the surface of the bundle on each side, is a thin strip of muscle near the base of the ribs. These strips extend cranio-caudally and measure 20 by 3 mm. On the left the strip lies over the third to eighth rib; on the right side it extends over the first to the sixth rib. The *serati posterior superior* are not identifiable.

The direction of the muscles of the anterior cervical region, as well as of those attached to the skull, is distorted with the underlying skeleton, but the muscles are well developed and not defective. Both sterno-cleido-mastoid muscles have normal origins and insertions. The two *splenii* arise bilaterally from fascia under the scapulae and are inserted normally on the mastoid process under the sterno-cleido-mastoid, and posterior to this on the occipital bone. The *longissimi* can be traced arising from the fascia over the cervical vertebral region and inserted on the mastoid processes. The *semi-spinalis capitis* muscles, arising from the upper ribs near their origin, are inserted on the occipital bone and are next to the deepest layer of musculature. The latter on each side consists of short fibers, rudiments of the short neck-muscles, the *recti*, and *obliqui*. More anteriorly most of the neck muscles are recognizable. The *digaster*, *stylohyoid*, *omohyoid*, and *sternohyoid* muscles are well developed. The *longus capitis* and *colli* are represented by a few strands along the anterior surfaces of the vertebral plate. The *scaleni medii* and *posteriores* are present as flattened bands of muscle arising in this region and inserted in the first and second ribs near their bases. The *scaleni anteriores* are symmetrical. They arise from the lateral processes of the superior cervical vertebrae and insert on the first rib near its center. The nerve trunks of the cervical and brachial plexus pass under these muscles and are tightly bound down by them.

Of the more anterior thoracic muscles, the *pectorales* are not disturbed. Both *serati anterior* muscles are defective and differ in their defects. On the right side there is more complete development. Here slips of the muscle arising from the distal portions of the first four ribs and from the eighth to the tenth ribs converge and are inserted around the inferior angle of the scapula. A few strands of muscle on the chest wall between the *pectoralis minor* and the *seratus* are present, which might be remnants of the latter. They are shaped like half a crescent, with fibers running longitudinally, and extend from the first rib, where they are 3 mm. broad, to the fifth rib, where they are 15 mm.

On the left side the serratus anterior is very imperfect. It is represented by a thin sheet of fascia, which originates from the first three ribs and is inserted in the scapula along the vertebral margin near the medial angle. A few scattered muscle-fibers, which also probably represent remnants of the serrati, arise over the fourth rib near its base and are inserted into the inferior angle of the scapula. The origin of the fascia and these muscle-fibers is shown in figure 21 by dotted lines. Some other muscles on the left chest wall, consisting of irregular projections from the dorsal bundle which covers the proximal part of the first seven ribs, may be serrati fibers which remained in their embryonic position close to the axis. Fibers which probably represent intercostal muscles pushed to the outer surface of the ribs are arranged along the lower border of the fourth rib. These extend onto the lower adjoining ribs. At the outer end they are 2 mm. across and near the base of the ribs they measure 20 mm. (See fig. 21.) Three small muscle bundles are situated at the distal end of the above-mentioned fibers.

The lateral and anterior abdominal muscles are well developed. Each rectus is 7.4 cm. by 3.2 cm. The right rectus has two inscriptions tendinae in its upper one-third opposite the sixth and seventh ribs.

To summarize: Those muscles which have undergone most disturbance are the trapezei, the rhomboidei, the serrati posteriores superiores, the serrati anteriores, and the sacrospinalis and short back muscles. The location of these muscular abnormalities, situated near the chief skeletal abnormalities, demonstrates still further that the pathological process is a rather sharply circumscribed one, limited to the neighborhood of the axis. The inclusion of the anterior serrati in this group does not contradict the statement, as the early anlage of the serrati is very near the axis.

The muscle disturbances of "monsters" have been but little investigated or recorded. From the scattered observations at present obtainable, any correlation is impossible.

VISCERA.

On dissection, the viscera are found crowded and somewhat distorted, but, with the exception of the right lung, are well developed. The thyroid is bilobed and measures 1.5 by 1 cm., the thymus measures 6 by 2.8 by 1.1 cm. The esophagus measures 4 cm. from epiglottis to cardia. The lesser curvature of the stomach is 1 cm. and its greater curvature 8 cm. The intestines are well formed. The appendix measures 8.5 cm. The colon is much bent upon itself. Because of unskillful handling the positions assumed by the rest of the intestinal tract were not ascertained. The pericardium, pleura, and diaphragm are intact. The heart is well developed. Sagittal section shows it cut through the right ventricle and left auricle. The valves are well formed. The ductus arteriosus is patent. The left lung is approximately normal; its lateral surface is shown in figure 23; it consists of two lobes and measures 3.9 cm. antero-posteriorly by 3.1 cm. perpendicularly by 1.7 cm. in its thickest medio-lateral diameter near the hilum. The right lung, side view of which is shown in figure 22, about equals the left in volume and is roughly a flattened cone-shape with apex directed anteriorly. Its corresponding measurements are 5.1 by 3.2 by 2.9 cm. It is formed of only one lobe. Along the margins four short fissures exist, directed toward the center; one 12 mm. in length

is situated on the posterior margin at the junction of the superior third with the middle third; on the inferior margin near its middle a similar fissure is situated, and halfway between it and the anterior end of the lung a shorter fissure 3.5 mm. long exists; on the superior margin another, 3.5 mm. in length, is present slightly anterior to the middle. These fissures are very superficial and extend for only a few millimeters on the medial surface of the lung. The relations of the bronchial tree were not determined.

The liver is flattened out horizontally and shaped like an L with the angle projecting anteriorly, the gall-bladder, which is 4.1 cm. in length, being situated on the inferior surface of the long arm of the L. The closed end of the gall-bladder lies near the tip of the angle and its long axis is directed diagonally toward the upper end of the latter. The spleen is 2.2 by 1.3 by 1 cm. The presence of the pancreas is determined histologically. It lies embedded in tissue near the vertebral column. Both kidneys and adrenals are somewhat compressed and distorted, the left much more than the right. The right kidney is somewhat flattened from side to side and at its upper end, and measures 4.5 by 1.2 by 3.5 cm. The right adrenal lies above it and measures 3 by 2 by 0.5 cm. The left kidney is bent upon itself and folded in with its closely adherent adrenal, so that together they form a rounded mass measuring 4.7 by 3.4 by 2.4 cm. The greater distortion of the left kidney and adrenal is very probably due to crowding, a result of the left-sided concave bending of the vertebral column in this region and fusion of the lower ribs on that side. The ureters and bladder are well formed. The uterus, tubes, and ovaries are well developed. Blocks of tissue of heart, kidney, liver, and adrenal were run through by the Levaditi method for spirochætes by Dr. Bullard, with negative findings. It is to be noted that the tissues had been kept in carbolic, which is not the fixation recommended for this method.

The developmental anomalies of the soft palate and the right lung are the most marked changes which have taken place in the soft tissues anterior to the vertebral column. They are both examples of arrested development and are secondary to, and probably the mechanical result of, the deformity of the vertebræ.

CRANIAL CAVITY AND CENTRAL NERVOUS SYSTEM.

The shape assumed by the cerebro-spinal cavity or subdural space is shown by figures 4, 5, 6, and 7 of the wax model. In figures 12 and 21 the model is given in its relation to the skeleton. The space consists of a shallow dome which contained the frontal and part of the parietal cerebral lobes. Below this dome a relatively slight constriction in the model denotes the enlarged foramen magnum (figs. 4, 5, 7, and 12). Under the foramen three rounded encephaloceles project posteriorly, and below these the pointed termination of the spinal canal may be seen. Situated ventral to the encephaloceles and continuous with them and with the base of the dome and the spinal canal is a blunt, wedge-shaped mass marked *w* in figures 4, 5, and 6. This portion fits into the pocket of bone formed by the thoracic and vertebral plate. With the exception of the inside of the large encephalocele, the space occupied by the central nervous system is lined with a continuous sheet of smooth dura. At the foramen magnum and in between the eminentiæ arcuatae of the tem-

poral bones and the sella turcica it is drawn into numerous folds. On the inside of the large encephalocele the smooth dural surface changes to a tissue composed of many blood-vessels, fibrin, and extravasated blood, as described in the beginning of the paper. The falx cerebri lies well over on the left side in its anterior and middle portions; posteriorly it ends in a single fold about the center of the superior margin of the occipital bone. The tentorium cerebelli is absent. The ventral surface of the subdural space is pierced by two rows of cranial and spinal nerves. These number 43 in all, 12 cranial and 31 spinal, the latter distributed as follows: 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 1 coccygeal. Both the anterior roots and the posterior roots with their ganglia are identified. In the cervical and thoracic regions they are extremely crowded.

The arrangement of the central nervous system is very complicated. The cerebrum lies part above and part below the foramen magnum. The cerebellum lies entirely below it. The medulla and cord lie bent and crumpled ventral to the other structures below the foramen. The surface of that part of the cerebrum which lies above the foramen shows definite fissure and sulcus formation. Below the foramen a large part of the contents of the encephaloceles consists of a hemorrhagic mass and much of the surface of this is covered with caked blood, so that only in a few places can typical cerebral surface markings be identified. Sections of these regions, however, show definite though rather undifferentiated cortical lamination.

From a brief study of sections of other parts of the left cerebrum, made by Dr. Charles Bagley, the following points are determined: The cortical structure for the most part is composed of a very wide granular zone, which is characteristic of the early and undifferentiated stage of lamination. There is, however, a prominent vertical fissure lying at the junction of the middle and posterior two-thirds of the cranial dome, which can be identified as the central fissure of Rolando; the ventral termination of this fissure rests on the eminentia arcuata of the temporal bone. The cortical tissue anterior to this fissure shows a definite lamination. There is a well-defined first layer; a broad and poorly differentiated second layer; the third layer is of medium width and is filled with small pyramidal cells; the fourth layer is inconspicuous, suggesting the agranular motor type; while the fifth layer is represented by large pyramidal cells, probably Betz cells. These cells are at least three times as large as any other cells which could be found in the cortex and it is owing to their presence and to the very narrow granular layer that this area is identified as motor cortex. Immediately posterior to this fissure there is a sharp change in lamination types. The large cells are lost and the fifth layer is represented by definitely shaped pyramidal cells of not more than one-third the size of those cells designated as Betz cells. Above this layer there is present a very heavy granular layer which is quite a contrast to the narrow granular layer of the area just described. It may be said, therefore, with a fair degree of certainty, that the tissue posterior to the fissure represents sensory cortex.

From the rather limited amount of material studied the only other localization that could be determined is that the cortical tissue pushed down on the side of the wedge-shaped mass (W in the model) represents subiculum. Here, passing from a cortex of quite deep extent, it becomes suddenly shallow and consists of a typical

first-zone layer containing few cells and a well-marked second layer with only a narrow zone of undifferentiated cortex beneath it. No calcarine type of cortex was identified, but the occipital lobe was probably included in the hemorrhagic mass in the encephaloceles, which was in such a bad state of preservation that no sections could be made.

On the right side of the cerebrum the fissure corresponding to the left central fissure of Rolando is situated slightly more anteriorly than on the left. The frontal lobes thus occupy most of the shallow vault and rest in the anterior and in the medial cranial fossæ as well, which latter normally hold the temporal lobes. The sulci of the frontal lobes are changed considerably from their normal positions. The anterior ends of the superior medial and inferior frontal sulci are bent very sharply downward. They are all situated nearer the base of the brain than usual, as if the tissue had been pulled down on the lateral surfaces. Thus the superior sulci lie halfway down the sides, with the medial and inferior sulci correspondingly below them. With the exception of part of the parietal lobes on each side, the rest of the cerebrum lies below the foramen magnum. The left-sided position of the falx cerebri allows more room in the cranial cavity proper for the right cerebral hemisphere than for the left. This results in a larger portion of the parietal lobe on the right side lying above the foramen magnum than on the left side, and correspondingly a larger amount of parietal surface lying below the foramen on the left than on the right side.

The cerebral tissue which lies below the foramen is partly contained in the large bony pocket formed by the upper vertebral plate, and partly in the middle and left encephalocele. Sulci are present over its surface, but are so distorted that they can not be identified, nor can the identity of the lobes be determined. In the contents of the middle and left sacs one can easily discern cerebral gyri, and the general histological structure of these is similar to that of the cerebral tissue situated above the foramen. In the middle sac there is a large amount of clot. The cerebral tissue which lies in the cervical and thoracic vertebral pocket is pressed out into a thin shell, and lies next the dura, being limited anteriorly by the emerging cranial nerves. On the sides and back it is continuous with the cerebral tissue lying in the cranial vault and with that pressed out into the encephaloceles.

In the interior of the brain the optic thalami may be identified, lying above the foramen. A small space representing the third ventricle, greatly compressed, lies between the thalami. Choroid plexus tissue is present. Its relations, however, to the adjoining structures could not be determined. The cerebral peduncles may be seen as flattened bundles lying central to the shell of the cerebral cortex. The optic nerves are present. The hypophysis lies embedded in the well-formed sella turcica. No other structures in this region or below can be identified until, in the pocket of bone formed by the thoracic vertebræ, the inverted floor of the fourth ventricle is recognized.

The midbrain with attached fourth nerves, the colliculi, and the aqueduct of Sylvius were not identified. The fourth nerves, however, were found at their dural exit. Judging from the position of the fourth ventricle floor, a sharp bend

with the angle directed posteriorly must have occurred in the midbrain region. At the beginning of the spinal cord a bend in the opposite direction is present. This bending of the brain stem and cord must have been in lateral outline shaped like a crudely drawn letter Z, as shown in figure 24, which is a diagram of various structures in the central nervous system near the midline. The surface of the inverted fourth ventricle floor is shaped like an isosceles triangle with its tip, which is its normal anterior end, directed backward toward the encephalocoels. The median sulcus is well defined. The tissue next to the median sulcus on both sides is slightly raised. The rest of the surface is flat. For estimating roughly the amount of distortion this fourth ventricle floor had undergone, a comparison of it with the fourth ventricle floor of three normal full-term fetuses was made. Each of the three showed a similar longitudinal ridge to be the extent of their surface markings. The main difference which this specimen showed seemed to be in a blunting of the posterior end which forms the base of the triangle already referred to.

From both lateral margins of the fourth ventricle floor cortical tissue resembling the flocculus is drawn backward, downward, and to the right, joining the cerebellar cortex contained in the right sac. It must be noted that while the flocculus is directed toward the posterior end of the specimen as a whole, it is drawn toward the end of the ventricle floor normally anterior. There is a much disturbed choroid plexus folded in with the cerebellar tissue. A fairly large amount of cerebellar tissue is present; part of this is drawn out into a sheet which is continuous with the flocculus and which passes posteriorly and to the right into the right encephalocoel, where it lies next to a rounded mass of cerebellar cortex. Bands of tissue connecting the cerebellum and cerebrum probably represent the brachium conjunctivum. There are smaller flat bands of tissue passing backward near the flocculus to the rounded cerebellar cortex which may have been remnants of the inferior cerebellar peduncles. There is no pontine enlargement.

From the tissue superior to and continuous with the floor of the fourth ventricle, the third and the fifth to the twelfth paired cranial nerves pass forward to their normal exits from the subdural space. They are elongated to between 20 and 30 mm. Their origin from the brain-stem lies opposite the first thoracic vertebra. In this region the beginning of the flattened cord can be made out, which is bent double upon itself. Some interest is attached to this Z bend of the brain-stem and cord. It seems to have been brought about in this case through traction on these parts by the major portions of the central nervous system slipping through the enlarged foramen magnum. Varying degrees of such kinking have been described. The condition in its milder forms has received the name of Chiari deformity, from a case described by Chiari in which the medulla is bent back over the cord for only a short extent. In Nageli's case of cyclopia there is a marked degree of such bending associated with splitting of the cord.

Caudal to the bend as a flat band the spinal cord extends to the level of the lumbar vertebra, where it terminates in a cauda equina. From its ventral surface the spinal nerves extend into the dura. At the level of the twelfth thoracic vertebra the spur in the vertebral plate has left an indentation on the flat cord and on the right anterior third of the inverted floor of the fourth ventricle.

The central canal, as such, is absent. Throughout the extent of the cord it is changed to a flat space following the contour of the vertebral column, whose floor is the cord and whose roof is partly the same cord inverted, partly the inverted floor of the fourth ventricle, and partly cerebellar tissue.

This fragmentary description of the central nervous system leaves much to be desired. It would have been especially desirable had we been able to present a clear picture of the relationships of the meninges. The main conclusion which can be drawn from its study is that the chief disturbance here evidenced is primarily one of distortion, rather than of absence or real lack of development of nerve-tissue.

CONCLUSION

The exterior alone of such a specimen as this certainly presents striking evidence that an organism can undergo most serious disturbances and yet maintain a definite though limited growth balance; but in order to ascertain in detail exactly what constitutes the limitations of this equilibrium more intensive study is necessary. A rather interesting series of anomalies is the result of such a study in this case. It may be noted that these anomalies are centered about the axis. The bony parts, the central nervous system, certain adjacent muscles, and overlying areas of integument share profoundly in this disturbance. Subsidiary disturbances of development are evidenced in a split soft palate and a one-lobed right lung. These facts, in addition to supplying a clearer knowledge regarding the individual specimen, contribute their small share in providing data for the better understanding of certain general problems of development. Classifications and analyses included in such subjects as osteology, myology, and organology can not be regarded as complete until they contain a comprehensive picture of teratological phenomena. This is almost entirely lacking at present. The teratological material has been so scanty that any satisfactory correlation of it has been impossible.

Up to fairly recent times teratology was considered an isolated science; it was thought that the laws applying to most natural phenomena were not applicable to its conditions, that it could not learn from or contribute to the normal sciences. Studies of the past half century have entirely reversed this view. Teratology today has for its basis the same fundamental sciences of chemistry, biology, and physics as has those sciences whose subject-matter deals with normal phenomena. It is constantly learning from these latter sciences, and in turn has been able to contribute suggestions on points of analysis or exposition regarding puzzling phases of normal development.

The necessity of furthering our knowledge regarding the etiological factors of specific abnormal conditions has been considered. Material at such an advanced stage of development as this specimen can contribute but little along this line. We can not determine by means of it the primary defect, nor again, except in a very general way, a chronological picture of the early processes. We must turn to embryological material and to other than morphological methods to obtain such knowledge.

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EXPLANATION OF PLATES.

ABBREVIATIONS.

anth.,	antherix.	m. obl. ext.,	m. obliquus externus abdominis (origin).
antitr.,	antitragus.	m. pect. maj.,	m. pectoralis major (origin).
b. occ.,	basioccipitales.	m. pect. min.,	m. pectoralis minor (origin).
c. i.,	first coccygeal segment.	m. quad. lumb.,	m. quadratus lumborum.
c. c. 7,	costal cartilage of seventh rib.	m. rect.,	m. rectus abdominis (origin).
c. eq.,	cauda equina.	m. rhomb.,	m. rhomboideus (insertion).
cer.,	cerebrum.	m. sacrospin.,	m. sacrospinalis (insertion).
cerebell.,	cerebellum.	m. ser. ant.,	m. seratus anterior (insertion).
cr. 2,	second cranial nerve.	m. ser. post. inf.,	m. seratus posterior inferior.
cr. 5,	fifth cranial nerve.	naev.,	naevus.
dep. em.,	depression made by eminentia arcuata of temporal bone.	r. I (rib),	right first rib.
epist.,	episternum.	r. hyp.,	right hypoglossal canal.
eust. t.,	eustachian-tube orifice.	rt. en.,	right encephalocle.
exos.,	exostosis.	s. I,	first sacral segment.
falx.,	falx cerebri.	sp. I,	first spinal nerve.
fl. IV,	floor of fourth ventricle.	sq. occ.,	squama occipitalis.
for. mag.,	foramen magnum.	st.,	sternum.
l. 1,	first lumbar segment.	tr.,	tragus.
l. 2,	second lumbar segment.	uv.,	uvula.
l. 1 rib,	left first rib.	v.,	vertex.
l. en.,	left encephalocle.	v. pl.,	vertebral plate.
l. pars lat.,	left pars lateralis.	w.,	central nervous system occupying bony vertebral pocket.
mid. en.,	middle encephalocle.	x.,	anomalous bone and its insertion.
m. lat. dors.,	m. latissimus dorsi (origin).	xyph.,	xyphoid.
m. lev. scap.,	m. levator scapulae (cross-section, fig. 17, insertion fig. 14).	iii.,	third ventricle.
		*	absence of soft palate.

PLATE 1.

- FIG. 1. Right lateral view of specimen shows extreme dorsal flexion with vertex level with shoulders. Middle and right encephaloclees show in this view. The distorted right ear here seen is drawn in detail in figure 8. ($\times 4$)
- FIG. 2. Superior view of the specimen looks directly at the face. Measurements given in table 1. Transversely across the forehead at the hair line an artefact puckering extends horizontally for 2 cm. ($\times 4$).
- FIG. 3. The dorsal view shows the shortened trunk, superior surface of head, and encephaloclees. ($\times 4$).
- FIG. 4. Right lateral surface of subdural cast, showing middle and right encephalocle. ($\times 4$).
- FIG. 5. Left lateral surface of subdural cast, showing middle and left encephalocle. ($\times 4$).
- FIG. 6. Ventral surface of subdural cast. ($\times 4$)
- FIG. 7. Dorsal view of subdural cast. The falx is seen to be to the left of midline. ($\times 4$).

PLATE 2.

- FIG. 8. Sketch of right ear (natural size), showing the antherix unusually prominent. The tragus lies relatively higher than normal, over rather than horizontally opposite the antitragus. The whole ear very narrow.
- FIG. 9. Sagittal section. Main outlines were geometrically projected and detail drawn free-hand. The viscera retain approximately their normal position. Absence of the soft palate is shown. The tip of the tongue lies over the left anlage of the split uvula. The vertebral column is bent and shortened and irregularly fused in its upper part. The arches of all the vertebrae are lacking. A fibrous band lies over the upper sacral vertebra, joining the opposing defective arches in that region and forming a short spinal canal. The section passes to the left of the sella turcica. The falx cerebri is seen well over on left side. The outline of the central nervous system, as is here shown, is used reversed for a diagram in figure 24. The section passes near the median margin of the left sac. ($\times 4$).
- FIG. 9a. Gives left side of bilateral anlage of uvula and orifice of eustachian tube. (Natural size).
- FIG. 10. Shows a dorsal view of the mounted skeleton, with scapulae in place. Varying degrees of gaping vertebral arches are shown at different levels of the spinal column. In the cervical and thoracic regions defective vertebral arches are fused together and markedly everted. In the upper lumbar region they are individually distinct, but still widely everted, while in the lower lumbar and sacral regions they are distinct and bent toward one another. The lumbar transverse processes and the sacral lateral processes are well developed and the coccyx of four segments is seen bent well to the left. In the lower thoracic region a cartilaginous spur projects dorsally from the vertebral bodies. All the thoracic and cervical vertebral bodies are fused together in a single plate. A slight lateral bending in this plate is present. The foveal surfaces of the atlas face the reader. The intervertebral foramina show large spaces in the lumbar region, which are a sharp contrast to the tiny areas of the contracted thoracic intervertebral foramina. On the right, the rough

surface of the tip of the first lumbar arch is shown, which joins the occiput; and on the left, the second lumbar arch, which does the same. Crowding of the base of the ribs may be seen, including the first to the sixth on the right and the fifth to the ninth on the left. The sternum is considerably to the left of the midline. A persistent episternum is present as a small cartilaginous knob, surrounding the manubrium. The irregular vertebral and superior margins of the scapulae are shown. On the left side the spicule of bone passes from the thoracic and cervical arches to the scapula. (Natural size).

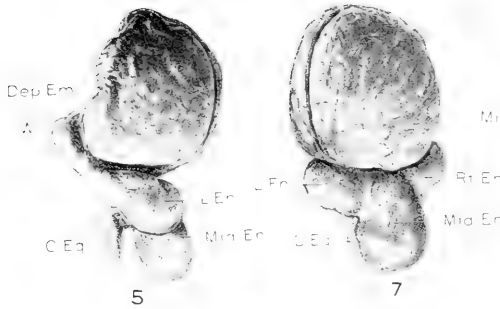
- FIG. 11. The superior view of the thoracic skeleton and the anterior surface of the cervical vertebral plate and of the occiput. In the cervical part, no vertebral bodies are distinct, but irregular radicular, and transverse processes project laterally from the central plate. The abnormal spicule of bone on the left side may be seen passing from the fused transverse processes to the left scapula. This view shows how the foveal surfaces of the atlas are shifted to the right in relation to their underlying transverse processes. The right fovea almost overlies the tip of the right transverse process, while the left fovea leaves the left transverse process uncovered. The left transverse process is bent up and joins the pars lateralis, thus forming a rather large foramen. The anterior surface of the occiput shows an asymmetrical oval outline pierced by a foramen, in its center. The double exit of the right hypoglossal canal shows. The irregular superior margins of the scapulae are seen. The episternum and the aborted second rib are demonstrated. (Natural size).

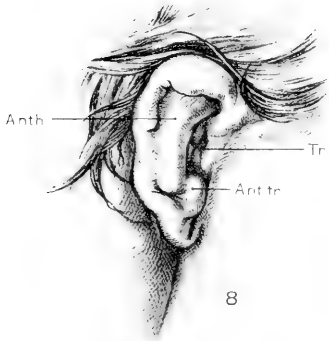
PLATE 3.

- FIG. 12. The left-hand view of the axial skeleton with subdural cast in place and median outline of specimen given. This figure shows the extreme dorsoflexion of the vertebral column. The occiput is in position and its squamosal junction on the left with the second lumbar arch is shown. The origin of the spicule of bone which projects out from the transverse process in the thoracic region is visible. The crowding and irregular arrangement of the fifth to the ninth ribs is shown. ($\times\frac{1}{2}$).
- FIG. 13. The superior view of the occipital bone shown with its enlarged foramen magnum. A normal-sized foramen is designated by a dotted line. The left jugular process is prominent when compared with the right, which seems to have been twisted over to the side. The anterior outlet to the right hypoglossal canal is shown with the tiny rod of bone which divides the exit of the hypoglossal foramen on the right side immediately under it.
- FIG. 14. The inferior surface of the occipital bone shows the large foramen. On the squamosal surface the exostoses which join the lumbar vertebrae show. On the partes laterales the condylar surfaces and on the left side the cartilaginous process which joins the transverse process of the atlas may be seen. The notched basal margin of the basiocciput also is visible. (Natural size).
- FIG. 15. This shows schematically the ventral surface of the thoracic vertebral plate with pairing of the origin of the seventh and eighth and ninth and tenth ribs on the right side, and of the first and second, and the third and fourth on the left.
- FIG. 16. This shows schematically the sternum with six costal cartilage attachments on each side. The last attachment on both sides is that of the seventh rib. The discrepancy occurs through the second rib becoming aborted on the right side, and the sixth being aborted on the left side. There are four centers of ossification on the midline of the sternum. The upper two are opposite the first costal cartilage and resemble an exclamation mark. The lower two are oval (4 by 3 mm.), with long diameter perpendicular. One is about at the middle point of the sternum and the other 1 cm. below it. An episternum surmounts the sternum and the xyphoid process projects at its inferior end.

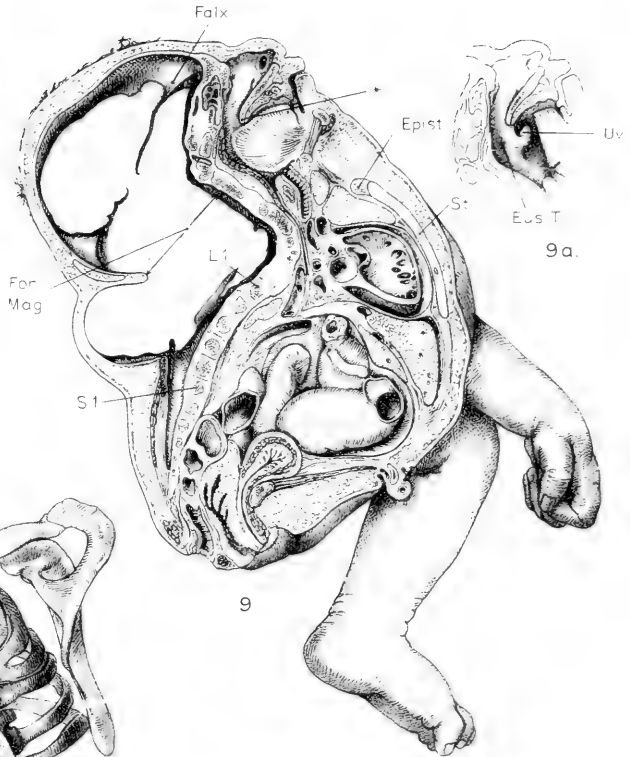
PLATE 4.

- FIG. 17. Dorsal superior view of a normal left scapula of a new-born.
- FIG. 18. Same view of left scapula of specimen 862a shows the irregular vertebral and superior margins with the abnormal spicule of bone attached. It also shows the sheets of fascia attached to the vertebral and median margins of the scapula and the insertions of the rhomboideus and levator scapulae muscles on this fascia. (Natural size).
- FIG. 19. Dorsal superior view of a normal right scapula of a new-born.
- FIG. 20. Same view of right scapula of this specimen, showing irregular vertebral margin. (Natural size).
- FIG. 21. Diagram of left thoracic and deep dorsal musculature on the left side of the mounted axial skeleton. The occiput and model of cerebro-spinal cavity are in place. The median outline of the specimen is also given in relation to these structures. Those muscles approximately normal are either sectioned or only drawn at their origin or insertion. They are the m. pectoralis major and minor, the rectus, the external oblique, the latissimus dorsi, the quadratus lumborum, and the levator scapulae. The abnormal muscles are shown entire, except for the serratus anterior, whose origin is indicated by broken lines. The largest mass of abnormal muscles consists of a longitudinal bundle extending from the sacrum to the occiput and labeled m. sacrospin. From about the center of this bundle the serratus posterior inferior projects onto the lower three ribs. The muscles at the upper end of the bundle are quite irregular. Along the fourth and fifth ribs a mass of muscle extends nearly to their costal cartilages. At the distal termination of these fibers lie several small irregularly placed bundles. In the upper thoracic region is a narrow band of muscle overlying the others. ($\times\frac{1}{2}$).
- FIG. 22. Lateral view of abnormal right lung formed of but one lobe. ($\times\frac{1}{2}$).
- FIG. 23. Lateral view of normal two-lobed left lung. ($\times\frac{1}{2}$).
- FIG. 24. Diagram of those structures of the central nervous system which lie near the midline and which can be identified. The outline of the subdural space used was obtained from the sagittal section. Posteriorly this passes near to the median margin of the left encephalocele. The cerebrum designated by a dotted field is shown protruding below the foramen magnum into the encephalocele. A small portion of the cerebellum, represented by line-hatching, is seen to lie very much flattened on top of the cord. The brain-stem and cord, much bent, are shown in solid black. Those cranial nerves which were identified are shown by lines. Only the first spinal nerve is shown. The floor of the fourth ventricle lies inverted on top of a flat cord.



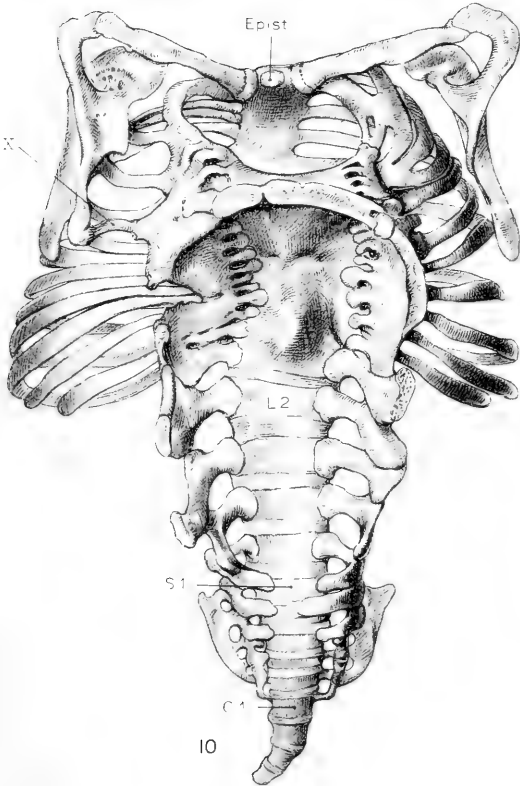


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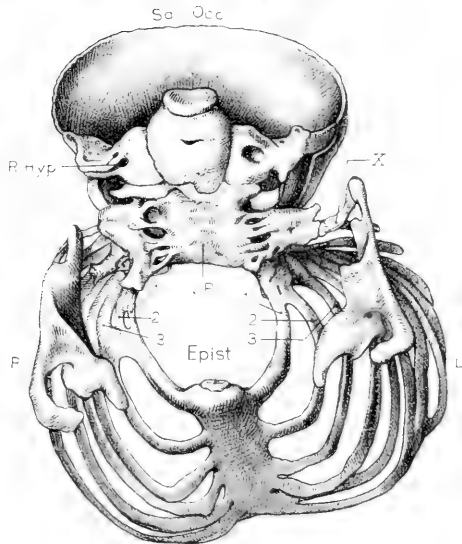


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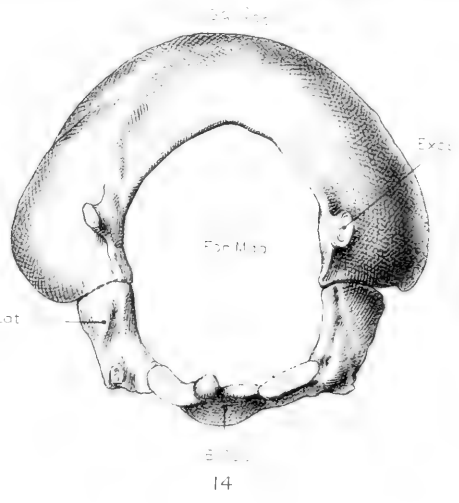
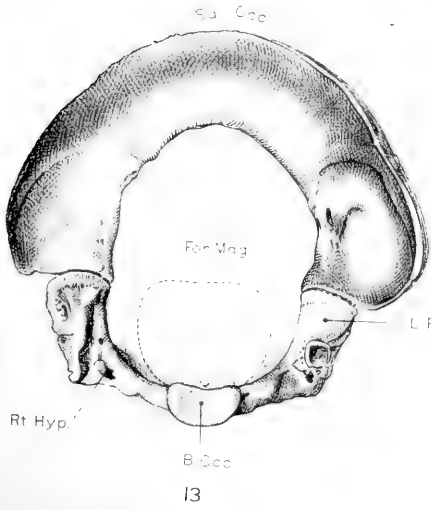
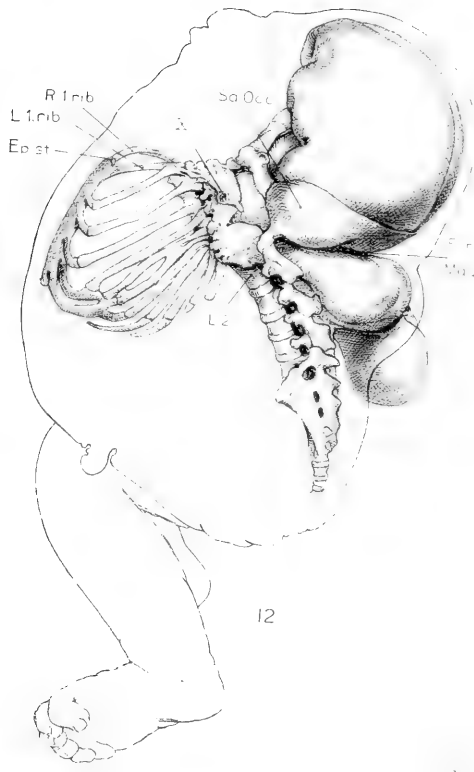
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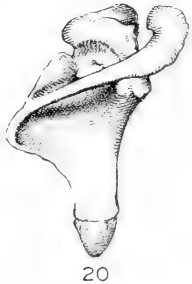
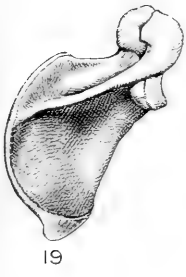


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CONTRIBUTIONS TO EMBRYOLOGY, No. 23.

A HUMAN EMBRYO BEFORE THE APPEARANCE OF THE MYOTOMES.

BY N. WILLIAM INGALLS.

With four plates and five text-figures.

A HUMAN EMBRYO BEFORE THE APPEARANCE OF THE MYOTOMES.

BY N. WILLIAM INGALLS.

The specimen which forms the subject of this paper came into my possession some time ago through the kindness of Dr. E. Peterka, of Cleveland. In the collections of embryology and teratology of the Department of Anatomy of Western Reserve University it is listed as embryo No. 1. On account of the very interesting and important stage of human development which it illustrates, a detailed investigation of its more essential features, especially as regards the embryo proper, has been undertaken. The extra-embryonic structures, chorion, body-stalk, and yolk-sac, and the evidence they offer on early blood and blood-vessel formation, will not be dealt with in detail at this time.

The intact ovum, when it came into my hands, had been for about a month in alcohol of unknown strength, but was, on account of its small size, quite well preserved. The following brief history accompanied the specimen:

- April 2. Intercourse (also about two weeks before?).
- April 8. Period expected; regular 24 to 26 days.
- April 14. Bleeding commenced, gradually increasing.
- April 17. Ovum cast off.

Before entering upon a discussion of the anatomical findings, something may be said as to the probable age of the specimen. Following the example of Bryce and Teacher (1908), which has been adopted so frequently, one can set up a similar table for the embryo in question:

Dimensions in millimeters.			Days elapsed from—			Age in days.	Remarks.
Ovum		Embryo (amniotic cavity).	Last period.		Lapsed period.		
External.	Internal.		Begin-ning.	End.	Begin-ning.		
9.1×8.2 ×6-6.5	ca. 1- 1.5 less	ca. 2.0	34	(?)	9	(17 to 18)	Abortion 15 (29) days after intercourse.

The estimated age of 17 to 18 days was put in parenthesis in the above table because we could not bring ourselves to look upon it with any very great degree of confidence. The figures were obtained by comparison with embryos which were obviously in a stage of development either more or less advanced and by reference to the recent estimates of Triepel (1914) and Grosser (1914). Triepel's suggestion of subtracting about 18 days from 34 in this case would give an age of about 16 days. Embryo No. 1 is far in advance of both that described by Fetzer (1910) and the v. Herff embryo of Graf Spee (1896), the ages of which have been given as 15 and 17 to 18 days respectively. On the other hand, it is distinctly less advanced than Frassi's

(1907) specimen, the age of which is estimated at (18) 19 days. The embryo Kl. 13 of Grosser (1913) is strikingly like our own, but we think a trifle more developed. Grosser gives the age as 19 days. A very similar stage of development is represented by the recent embryo of Strahl (1916), but concerning which there are no data as to age.

If one again adopt the method of Bryce and Teacher, it is possible to determine when, as regards the menstrual cycle, fertilization took place. The duration of the menstrual cycle in this case may be taken as 25 days—regular 24 to 26—and if we let the age of the embryo be 18 days, then fertilization occurred on the seventeenth day of the previous menstrual month. Such a date would harmonize very well with the findings of Fränkel on ovulation, as interpreted by Grosser. The time thus assigned for fertilization could easily be pushed still farther toward the beginning of the menstrual month by either supposing that the embryo is more than 18 days old or that development was arrested by the hemorrhage some time before abortion occurred. As regards the last point, we see no reason to suppose that development stopped very long before the ovum was expelled. Fertilization on the seventeenth day of the menstrual cycle would mean that the intercourse of April 2 could not be considered in computing the age, since it falls on the twentieth of the cycle, 15 days prior to the abortion.

The history of intercourse two weeks before the one just noted is, however, subject to doubt; it would have occurred on the sixth day of the cycle. This would have called for a rather protracted sojourn of the spermatozoa within the tube—namely, 11 days, a period over which they are doubtless quite capable of retaining their fertilizing power. The sixth day of the cycle would fall about the beginning of the so-called period of œstrus, and, in view of the reputed increase in libido at this time and of certain obstetrical experiences, one may suppose that not only is this a favorable time for insemination, but also favorable to a prolonged stay of the spermatozoa within the tubes.

The arrival of the ovum in the uterus and the date of its implantation are dependent upon the unknown factor of the time consumed in traversing the tube. (Grosser points out that this may vary, depending upon tubal (menstrual) conditions, and he is inclined to raise the estimate of 10 days, given by himself and Triepel, to 14 days or even more. In either case, implantation would have occurred after the beginning of the lapsed period, and some influence other than that of the actual ovum upon the uterine mucosa would have to be invoked to inhibit the impending menstruation. Such an influence, as is well known, has been sought in the tiny ovum within the tube, acting alone or in conjunction with the newly formed corpus luteum. Assuming 10 days as the period of migration (7 days in the table of Bryce-Teacher), implantation would have occurred in our specimen on the second day of the cycle. It would therefore have found a mucous membrane especially adapted to its nutritional needs, possibly thus accounting for its large size as compared with the stage of development; but, on the other hand, the inhibitive action upon this same mucosa, from whatever source, may have come too late to save it, as seems also to have been the case with the Bryce-Teacher ovum.

It is not the purpose of this paper to enter into any discussion of the relations between ovulation and menstruation, the passage of the ovum along the tube, or other mooted questions which may have a bearing on the age and development of embryos, but a few words might be said regarding what seem to us to be certain aspects of this subject. Of the three cardinal embryonic features so often quoted, viz, age, size, and stage of development, only the last has any great practical importance, and even here there is more or less variation in different parts of the embryonic body. The fact that age, size, and development by no means run parallel has been pointed out very clearly by Mall (1914; *cf.* also Rabl, 1915), and indeed there is no reason to expect that they would be exactly comparable. It seems to us that with ova of the same age, dating from the time of fertilization, discrepancies in their size and development may, and not without reason, be assigned to different environmental factors. Tubal conditions during migration, varying at different times in the menstrual cycle, might play a certain rôle, resulting in more or less rapid progress along the tube, accelerating or retarding development. Conditions in the uterus at the time of implantation, premenstrual, menstrual, or postmenstrual, etc., time of ovulation, or conceivably the actual size or potentiality of the ripe unfertilized ovum and other unknown or unappreciated factors, might bring about the variations so often observed. The relative independence of age, size, and degree of development is most strikingly evident in the case of pathological ova.

The variation in size at the same developmental stage is especially marked in the case of embryo No. 1 (see page 116). The chorionic vesicle is roughly of the same dimensions as that of the embryo Kl. 13 of Grosser or of the embryo of Eternod (1898), while the blastoderm of No. 1 is, as well as can be determined, about twice as long as in Grosser's case—but of about the same stage of development and a half longer than in Eternod's—but far less advanced. In other words, the size of the vesicle is fairly commensurate with the assigned age, while the embryo is disproportionately large, both as regards the chorionic sac and the estimated age; it is, in addition, very large, considering the degree of development. The opposite disproportion between the sac and contained embryo is quite characteristic of pathological ova, and it may be that we are dealing here with the results of some subtle influence which has stimulated the growth of the embryo proper without, however, having disturbed unduly its orderly development or brought down the balance on the pathological side. A retardation in development but not in growth might account for observed conditions. In the face of the extensive literature on the early chapters of human development we can not claim to present a typical embryo of the middle of the third week, but simply a normal specimen of about that age. It is difficult enough to find one's way among the unnumbered variations of adult morphology, but as regards the embryo we have hardly scratched the surface. It would not be surprising if we had before us in this specimen one of those examples of embryonic variation which are so abundantly present later on (tail, pronephros, milk-ridge, fifth aortic arch, etc.). For obvious reasons these individual variations become more plentiful as development proceeds, but at no time need they occasion any surprise and always may they be ranged under the same rubric.

One carries away from the perusal of the literature bearing on the age of young ova the relations between ovulation, menstruation, fertilization, implantation, etc. the impression that the actual age of a normal embryo has a value, for purposes of classification at least, more apparent than real if not in large measure fictitious, and the more so because this assigned age can be only a more or less defensible approximation. Complicating the more general factors touched upon above are the individual variations and peculiarities, pathological states it may be of the maternal organism if not also of the future ovum, temporary bodily or seasonal conditions and the like, not to mention possible paternal influences, a variety of factors which it is difficult or impossible to evaluate, and we are imperceptibly carried into problems of fecundity, absolute and relative sterility, and other clinical, racial, and sociological questions. In the end one can appreciate the perplexity of Hyrtl when he wrote long ago in his characteristic vein: "So weit wäre nun Alles recht. Nur begreift man dabei nicht, warum die Frauen nicht fortwährend schwanger sind, und aus dem Schwangersein ihr Lebelang nicht herauskommen."

The entire specimen was stained in bulk with hematoxylin, and after sectioning at 10 microns was counterstained with eosin-safranin. The plane of section, which it was supposed would be transverse to the embryo, the interior of the vesicle having been examined somewhat before embedding, came out quite obliquely, as can be seen in the various text-figures. While the staining reactions are not always what could be desired, still there is no doubt that the essential features have been preserved. Occasional mitoses are in evidence, as will be noted later.

I. THE CHORIONIC VESICLE, GROSS.

The following account is taken from our notes made soon after receiving the specimen. The intact vesicle (plate 1, figs. 1 and 2) is quite regularly formed and distinctly flattened; the surface showing the circular area of free villi is slightly more convex than the opposite. The form tends to be roughly quadrangular with the corners rounded off. To the touch the vesicle feels quite firm and resistant. Measured under a magnification of 5 diameters, the ovum shows the following dimensions: length 9.1 mm., breadth 8.2 mm., thickness 6 to 6.5 mm. The internal measurements are from 1 to 1.5 mm. less.

One surface of the ovum presents a large, sharply defined area of free chorion and its villi, situated at one end and extending about to the middle. These villi vary greatly in size and shape. They may assume the form of long, slender processes or of thick, broad, irregular masses, often in clumps together and leaving a few small areas free. There are a few straw-colored areas as from blood-stains. The remainder of this surface of the ovum is smooth and varies in color from a straw through a red (fresh meat) to almost a purple.

The opposite side of the ovum is much smoother, covered partly by a much thinner layer of maternal tissue through which project more or less freely the villi of the chorion. These villi appear to be rather more pointed and slender than those previously noted, resembling papillae filiformes. There seems to be no part of the sac which does not possess villi.

Upon making an incision along one side of the sac to facilitate embedding, a large cavity is found into which projects the embryonic anlage attached to the side showing the free villi. Regarding the embryo proper nothing more than a small, whitish, globular mass (yolk-sac) can be made out for fear of injuring the embryonic structures. At this time there were seen a few minute but distinct strands traversing the cavity (exocoelom) and connecting the inner surface of the vesicle with the yolk-sac. Traction upon the margins of the opening in the vesicle could be seen to have a very distinct effect upon the yolk-sac on account of the attachment of the above-mentioned filaments.

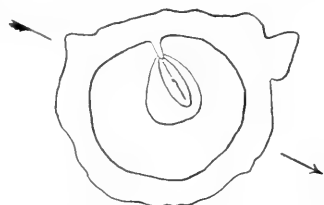


FIG. 1.—Schematic representation of embryo and vesicle. \times about 4.5. From photograph, plate 1, fig. 1, and reconstructions. The arrow indicates the line of section.

II. THE EMBRYO AND ADNEXA.

The main features of the embryonic anlage are shown in the text-figures 1, 2, and 3 and in the photographs of the model (plate 4, figs. 1 and 2). The general shape of the blastoderm is not unlike that of the Frassi embryo, but narrower and as a whole very much larger. Its dimensions, determined on the reconstructions ($\times 100$), are 2 mm. in extreme length by about 75 mm. in breadth at the widest point. The ventral surface of the embryonic disk presents a very slight ventral concavity in the sagittal plane, while at right angles to this the same surface is for the most part convex, *i. e.*, projecting slightly into the yolk-sac. The dorsal surface is in general more strongly convex, owing to the presence of the prominent ectodermic folds. The amnion above completes roughly the curvature of the yolk-sac below. The anterior extremity of the blastoderm is quite regularly rounded and, especially on the left side, is undermined by shallow extensions of the exocoelom; the posterior half tapers evenly to a point.

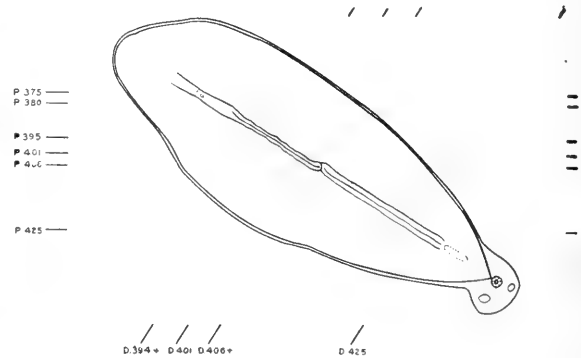
As may be seen in the dorsal view of the model, the appearance of the posterior third of the embryonic disk is quite different from that of the middle and anterior thirds. This posterior part contains the cloacal membrane and about the caudal half of the primitive streak and is the most regularly formed part of the entire blastoderm. The dorsal surface presents here, on either side of the median line, two rather steep, even slopes (plate 3, fig. 1), the left slightly more extensive, which extend from the region of the cloacal membrane and primitive streak to the attachments of the amnion laterally. The primitive groove appears in the model simply as the central, deepest portion of this valley-like area. The ventral surface of the region in question is strongly convex from side to side. Distinct primitive folds can not be made out.

The central third of the embryo includes the anterior half of primitive streak and the head-process region in front of it. Most conspicuous here are the two large folds of ectoderm which extend from near the middle line to the attached border of the amnion; the fold on the left side is broader and more regular than that on the

right. Separating these prominent folds (plate 3, figs. 2 and 3) lies, in their caudal halves, the anterior end of what we may call the primitive groove, here very deep and narrow. The groove between these folds in their cephalic portion is much shallower and finally lost. Near the posterior end of this shallower groove, which is continued forward without distinct interruption from the primitive groove, but in a plane slightly to the right, lies the minute dorsal opening of the archenteric canal—so-called chordal canal—slightly to the left in the bottom of the groove.

The anterior third of the blastoderm is in general slightly convex, but its surface is broken up by many small, irregular folds to which one can attach no significance. It is certain that the ectoderm in the anterior half of the embryo has suffered more distortion than any other part. The result has been an obliteration, as far as they may have been indicated, of the early medullary folds anteriorly, coupled with what seems to be their accentuation and prolongation posteriorly. That these last-mentioned folds, occupying the center of the blastoderm, have anything to do with the medullary folds is, considering the stage of development, very doubtful. The posterior ends of these folds, especially on the right, have a remote resemblance to the caudal lobes of a later date.

FIG. 2.—Blastoderm and axial structures in dorsal view. X about 40. For more exact details of the head process see text-figures 4 and 5. The location of the cloacal membrane is indicated by dotted outline. Close to the posterior tip of the amnion is the allantois; in section and near it, in the body-stalk, are sections of two vessels. The marginal lines have reference to the location from which were taken the photographs (P.) and drawings (D.) shown on plates 2 and 3.



The amnion lies close to the embryonic ectoderm anteriorly, while farther back it is lifted high above it by being incorporated in the body-stalk. Any indications of the presence or recent disappearance of an amniotic duct as noted by Grosser (1913) and Strahl and Beneke (1910) are wanting. This amniotic duct may very well be one of those instances of embryonic variation referred to above, not only variable but quite possibly very transient, and in this same category may be placed a peculiar feature of our embryo to which we would here draw attention. As can be seen in the accompanying illustrations, the caudal tip of the amnion lies in very close proximity to the allantois, a short distance above the connection of the latter with the yolk-sac. In at least two sections there is a very distinct though tiny, narrow outpocketing of the amniotic cavity toward the allantois (not shown in the figures). Here the epithelium of the amnion is of a low cuboidal type in contrast with its squamous character in the immediate vicinity. There is no connection

between the cavities of the amnion and allantois, but their epithelial cells fuse into a single mass over a small area of contact. The lumen of this amniotic diverticulum, which is also very short, is only a few microns in diameter, tapering slightly toward the allantois. This structure may well be compared with the secondary connection set up between the same cavities in certain reptiles, the *canalis amnio-allantoideus* of Strahl (Schauinsland, 1902).

Histologically the amnion is composed of two layers of cells which are generally frankly squamous. The transition of the ectodermic cells to the flattened type is as a rule quite abrupt. In certain places, however, the cells, near the attached border of the amnion, are cubical and become squamous only at some distance from the line of reflection. The cells of the mesodermic layer of the amnion have slightly smaller, more densely staining nuclei and seem to present a clean, even surface to the exocoelom. Throughout most of the membrane its two layers are in close contact, often almost indistinguishable, but along the borders there is frequently a considerable space between the two, across which run numerous irregular cell-processes connecting the ectoderm and mesoderm but apparently belonging rather to the latter. Scattered mesodermic cells in the interval between the layers are quite common. Posteriorly, where the membrane is cut tangentially, the ectodermic elements appear polygonal in outline, with large, pale nuclei which almost fill the cell.

In the consideration of the embryo proper we shall begin with the most posterior structures and gradually work forward. In like manner we shall endeavor to separate the following observations from the speculations which they invite.

Mention has already been made of the indications of a *canalis amnio-allantoideus* in the caudal extremity of the amnion. A short distance in front of this, in the axis of the blastoderm and at the posterior end of the primitive streak, lies the cloacal membrane. As shown in figures 2 and 3, it measures about 0.12 mm. in length. This measurement and likewise the figures are at best approximations, maximum limits in any case, since it is difficult to determine the exact line, if there be one, between the membrane and the primitive streak. There is in the cloacal region a well-defined groove in the ectoderm, less conspicuous, however, than the primitive groove with which it is directly continuous. In certain sections it is quite evident that the ectoderm and entoderm are in immediate contact, the mesoderm being at some little distance. In other sections, largely on account of the irregular lower surface of the ectoderm, the picture is very much like that of the primitive streak. The conditions found here in the cloacal membrane are such as would be expected from the gradual and not entirely regular transformation of the streak into the membrane. All that is required is an arrest of mesoderm formation and the subsequent separation of the upper and middle germ-layers. The entoderm below is a perfectly distinct layer the cells of which have nuclei larger and paler than those of the other layers. The condition of the ectoderm is such that the real character of its cells can not be made out. Its free surface is of course distinct, but the lower surface is often markedly irregular and frayed out. In the region under discussion at present it is undoubtedly of the columnar type, in most places, if not everywhere, pseudostratified with one, two, or occasionally three layers of

nuclei. Farther back and laterally the ectoderm is frankly one-layered, with low columnar or even cuboidal cells. Throughout most of the embryo, however, the arrangement of its nuclei in several layers, the character of its lower surface, and its often irregularly varying thickness make impossible any definite statements as to its real structure. Cell boundaries are not commonly visible.

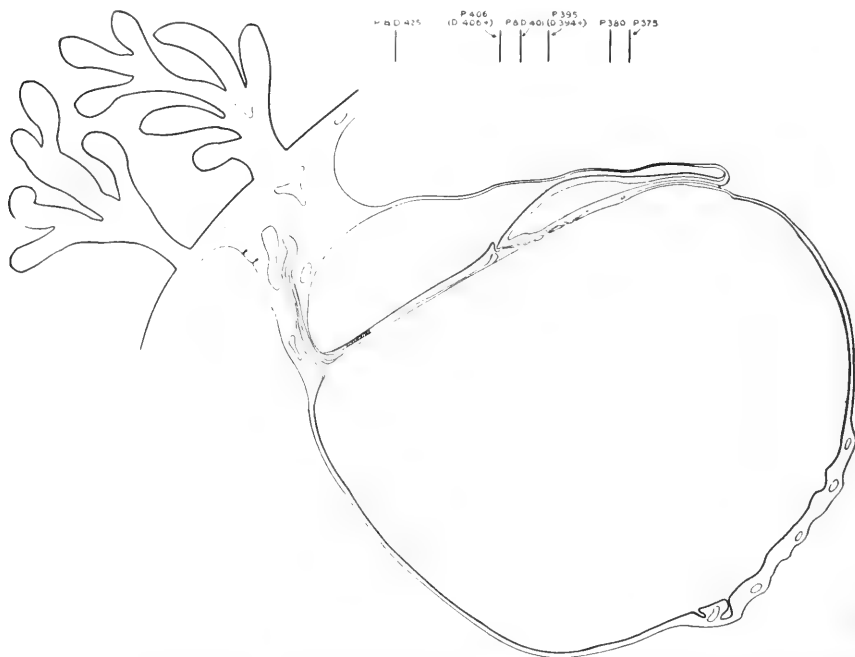


FIG. 3.—Median sagittal section of embryo, yolk-sac, body-stalk, allantois, and adjacent chorion, slightly schematized, about $\times 40$. The primitive streak and head process are represented as if both lay in the same plane. The chorionic villi are quite diagrammatic, their connections with each other not being indicated. Two "funnels" in the body-stalk, further details in text. Marginal lines as in fig. 2.

The primitive streak (figs. 1, 2, and 3; plate 2, fig. 1; plate 3, fig. 1) is very long in this embryo, making up about one-third of the axis of the blastoderm, the center of the latter being just in front of the anterior end of the streak. Its length, measured from the anterior limit of the cloacal membrane to the dorsal opening of the archenteric canal, is about 0.65 mm. In position it is not exactly central, but is displaced a trifle to the right. The primitive groove is well defined throughout, the continuation, as noted above, of its anterior end passes slightly to the right of the opening of the archenteric canal and the beginning of the head process. This condition, in which the primitive streak and its head process do not lie in the same sagittal plane, is not uncommon in a variety of forms, and apparently the head process is usually on the left, as in this case. The anterior limit of the streak is of course

easily determined, and our reason for assigning the posterior limit is that at this latter point there is not only a conspicuous connection of mesoderm and ectoderm, but also a rather sudden separation of the entoderm from the cell-mass just above. This cleft between the entoderm and the primitive streak is present throughout its caudal half, while anterior to this the entoderm lies very close to the newly formed mesodermic elements.

The primitive groove is best defined at its posterior end, where it appears as a sharply outlined groove between the more gentle slopes of the ectoderm on either side. Farther forward, where the high ectodermic folds are found, this fine median furrow can not be distinguished, being simply the bottom of a deep, narrow trough. In its most caudal part the primitive groove possesses a narrow, flat floor bounded by perpendicular walls of distinctly greater extent. The breadth of the floor may be estimated at about 0.01 to 0.015 mm. This dimension is accentuated in the photographs on account of the obliquity of the sections. Followed forward, the groove varies in shape and width, its floor soon disappears, and it is finally lost in the general slope of the embryonic ectoderm. In the primitive groove the outlines of the ectodermic elements, free surface, and cell boundaries are more distinct and the arrangement of the nuclei is rather more regular than elsewhere.

In those places where there is a well-defined floor one can sometimes see, in one side of the floor, a deep, distinct secondary groove, usually on the right. A few sections show a definite lipping of the primitive groove, *i. e.*, the lateral wall; this being observed only on the left side, bulges into the groove, forming a small recess between the floor and the wall. These peculiar conditions occur only near the posterior end of the primitive groove. The ectoderm of the groove presents 2 to 3 or 4 layers of nuclei and is thickest in front. The floor is often composed of but a single layer of low cells, remarkably thin in places but always intact.

From the walls of the primitive groove, much less conspicuously from its floor, where this is well marked, the ectoderm is continued directly into the mesoderm lying laterally, while the floor of the groove, often much thinner than the side-walls, is widely separated from the entoderm, in which space occasional free cells may be seen. There are in a few places interruptions in the transition from ectoderm to mesoderm, probably artefacts due to the loose character of the latter layer. The width of the primitive streak, *i. e.*, of the zone of proliferation of mesoderm, is about 0.05 mm. Towards its anterior end this zone gradually becomes more massive, the connection between ectoderm and mesoderm more extensive, and the entire median line between ectoderm and entoderm becomes filled with closely packed mesodermic cells. At the very anterior end of the streak, just behind the primitive node, the lower germ-layer is again widely separated from the cells dorsal to it. Everywhere, however, in the primitive streak can the entoderm be seen as a distinct, definite cell-layer.

The mesoderm on either side of the middle line forms a well-defined, loosely cellular stratum of slightly varying thickness. It is continuous laterally and posteriorly with the outer layers of the yolk-sac and amnion, anteriorly with the mesoderm on either side of the head process, and mesially with the ectoderm of the primitive

streak. This layer of cells is thinnest behind and lies uniformly close to the ectoderm, to which its cells are attached by numerous fine processes. The constituent cells vary considerably in size and shape; most of them possess a number of larger or smaller, partly anastomosing processes, while some seem to have a smoothly rounded cell-body. One finds quite generally a fine, sharp line on that surface of the mesoderm toward the ectoderm, very much like a basement membrane of connective-tissue origin, the *membrana prima* of von Spee. There is no indication anywhere of an arrangement of the mesodermic cells in two layers, as has been repeatedly described in the primitive-streak region.

The majority of the mitotic figures observed in this specimen are found near, or at a short distance from, the primitive streak; by far the greater number of these occur in the ectoderm and mesoderm, especially in the former; only rarely are they

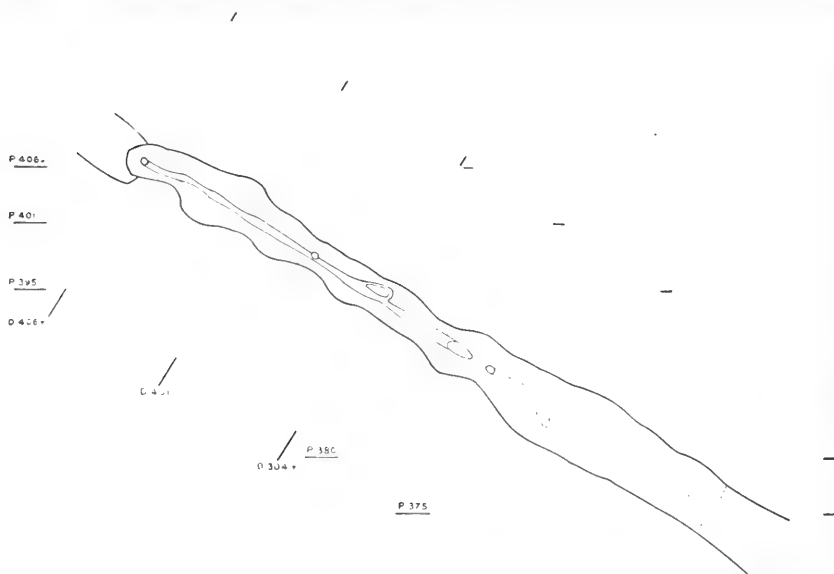


FIG. 4.—Head process and completion plate. Dorsal view, but in the plane of the sections (*i. e.*, some foreshortening. Cf. fig. 3). On the left is the anterior end of the primitive streak; immediately anterior to this is the dorsal opening of the archenteric canal. The four ventral openings of the canal are seen in the center of the figure; dotted lines indicate that the lumen is indistinct or doubtful. The small dotted ring near the anterior end represents a very doubtful cavity, and just beyond this is shown the line where the entoderm becomes a distinct layer. Marginal lines as before.

seen in the entoderm. In those cases in which the axis of the spindle can be determined it is found in nearly all instances parallel to the surface of the ectoderm or mesoderm and at right angles to the median line.

As mentioned above, the anterior part of the primitive groove is but the bottom of a deep, median furrow in the blastoderm. This furrow becomes rapidly wider and

is continued some distance farther forward, but distinctly to the right of the head process, where it gradually fades out. Here, at the anterior end of the primitive streak, is the primitive or Hensen's node. There is, strictly speaking, no real node, knot, or distinguishable enlargement at this point, and nothing to indicate any separation between the groove and the archenteric canal. Immediately caudal to the beginning of the canal the ectoderm becomes thinner and there appears a wide interval between the ectoderm and the last of the primitive streak dorsal to it.

Lateral to the node, or better in it, *i. e.*, in the walls of the first part of the canal, the ectoderm and mesoderm are in broad connection. Just anterior to the node the head process has freed itself from the overlying ectoderm, is continuous with the mesoderm on either side, and fused with the entoderm below. The posterior ectodermic opening of the archenteric canal is very minute, being only about 0.005 mm. in diameter (plate 2, fig. 2; plate 3, fig. 2). The actual opening on the surface can hardly be made out, since it is bounded only by the slightly staining cytoplasm of the surrounding cells, the nuclei of which, in contrast to other regions, are here at a

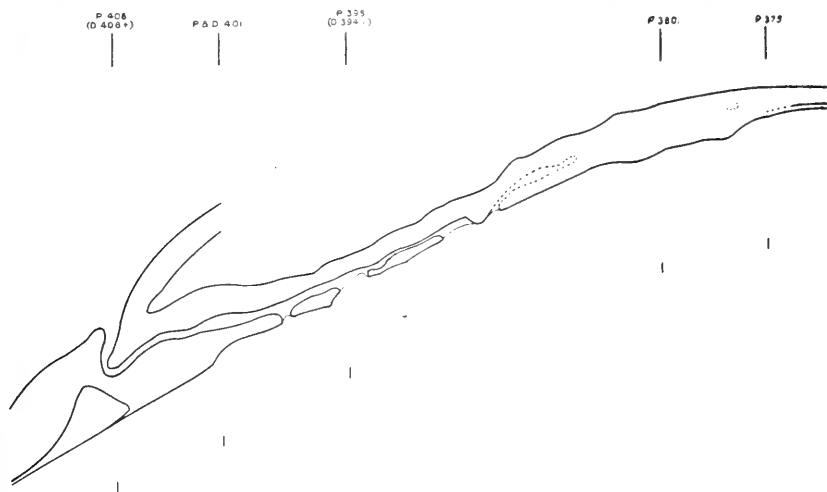


FIG. 5.—Head process and completion plate. Right lateral view. The primitive streak, on the left, and the posterior end of the archenteric canal are represented as being in line with the more anterior structures. Other explanations under fig. 4.

greater distance from the free surface. From this point the canal passes directly ventrad through the substance of the primitive node, turns forward and to the left, and again forward in the line of the head process (figs. 2, 3, and 4). It is at the node, and here only, that the three germ-layers are fused with each other (plate 3, fig. 5).

Extending cephalad from the primitive node in the axis of the blastoderm is the head process of the primitive streak (figs. 1 to 5). This structure, including the completion plate in front, is slightly longer than the primitive streak, measuring about 0.75 mm. in length; its diameter is variable, but in general gradually increases from behind forward. The posterior half, or head process proper, varies in width from 0.03 to 0.05 mm., its lumen from 0.006 to 0.01 mm., while the length of its lumen, the archenteric or canal of Lieberkühn, is 0.34 mm. The average breadth of the completion plate is about 0.06 mm.

The head process is an axially placed, hollow, cylindrical mass which, at its origin in the primitive node, is directly continuous with the superficial ectoderm and the substance of the primitive streak, as well as with the mesoderm on either side. It very soon becomes free from the ectoderm above and fuses with entoderm below; its lumen, which is at first nearer the dorsal surface of the process, takes up a central position, while at the same time the dorso-ventral diameter diminishes somewhat. In considering this structure we shall begin at its posterior end, at the point where it has just disengaged itself from the surface ectoderm. It appears here in section as a roughly pyramidal or wedge-shaped mass projecting well into the space below the ectoderm. Sharply limited above, this mass is fused at its base with the entoderm and mesoderm. The lumen is yet eccentrically placed; the cells dorsal to the lumen are much fewer in number, more deeply staining in their cytoplasm, and have a more epithelial arrangement than those between the lumen and the yolk-sac. These latter cells are much more numerous, more irregularly massed together, and are quite indistinguishable from the neighboring mesodermic and entodermic elements. A few sections in advance (plate 2, fig. 3; plate 3, fig. 3) the head process is rather lower and distinctly broader, its free outlines more curved, while its cavity has increased in size and lies about the center. The cells which bound the archenteric canal dorsally are frankly epithelial; their nuclei are nearer the base of the cells, while the cytoplasm is deeply stained.

The cells ventral to the lumen show no definite arrangement; they stain only faintly and no layer of entoderm can be made out beneath them. The mesoderm is directly continuous with both these groups of cells, but rather more definitely, on account of their staining reactions, with the cell-mass below the canal. The ventral surface of the head process is, near its posterior end, concave from side to side and at about its margins the entoderm can be recognized as a separate cell-layer. The thinning out and eventual loss of the floor of the canal is apparently due to the rearrangement of the cells here (*cf.* fig. 5), there being no evidence of a corresponding loss or destruction of cells. We have in these two distinct cell-groups, dorsal and ventral to the lumen of the head process, the plaque notochordale and plaque lécithoentérique respectively of van Beneden (1899). To these we shall take occasion to recur later. Near its posterior end, where the lumen is more dorsally placed, the cells of the plaque notochordale are only about half as numerous as those of the plaque lécithoentérique; at the point shown in the illustrations they are approximately equal in number.

A little anterior to the sections just mentioned is found the first ventral opening of the archenteric canal. It is very small, located entirely in one section, and appears as a narrow passage connecting the canal with the cavity of the yolk-sac. Just beyond this is the second ventral opening (plate 2, fig. 4; plate 3, fig. 4), very large, mainly on the left side, and limited dorsally by the beautifully epithelial notochordal plate. The epithelial cells of the plate are here columnar, their nuclei are slightly nearer the base than the free surface, and their cytoplasm stains rather intensely. In spite of their character these cells are not to be separated laterally from the adjoining mesoderm and entoderm. In the sections which follow, the notochordal plate varies considerably both in breadth and distinctness. The canal likewise is not always well defined; its floor varies in thickness or may even appear deficient. It can be traced forward, however, to the third and last definite ventral opening, where again the notochordal plate is very conspicuous, while in the floor appear a few cells, some of which may be free. This last portion of the head process hardly projects above the level of the neighboring mesoderm.

Anterior to the head process and continuous with it lies the so-called completion plate, the *Ergänzungsplatte* (des *Urdarmstranges*) of Bonnet. Its posterior limit may be placed just in front of the third and most anterior opening of archenteric canal, while at its opposite extremity it is gradually lost as the two lower layers of the blastoderm becomes distinct. Its length, with the limits just noted, may be taken as about 0.4 mm.; its width, averaging about 0.06 mm., is greater than that of the head process proper. In structure it differs very markedly from the typical head process just described. The transition between the two appears to be gradual, at least so far as can be determined on a transverse series. On following the sections forward it is seen that the conspicuous dorsal cells (notochordal plate) rapidly lose their epithelial character, and the lumen (which was such a prominent feature before) becomes very doubtful if not actually wanting (indicated by the dotted outlines in figs. 3, 4, and 5, solid line in fig. 2). At the same time there is a gradual but not uniform increase in the breadth and thickness of the plate until it reaches nearly twice the dimensions of the head process, bulging below into the yolk-sac and above into the space between the ectoderm and mesoderm. Along its lateral borders, which are never sharply marked, it is directly continuous with the mesoderm, as this layer is with the head process farther back. At its anterior end it is gradually lost in an ill-defined, axial condensation of mesoderm, and very soon this also disappears. Structurally the completion plate is made up of a rather closely packed mass of cells in which no details can be made out. The entoderm beneath does not lose its identity to quite the extent which it does in the head-process region, but still can hardly be recognized as a distinct layer. Toward the anterior limits of the plate the entoderm appears as a definite layer of large, thick, almost cuboidal cells. At certain points there are indications of a sort of doubling in the plate, due to the presence of a shallow furrow on its dorsal surface. Here, and also where this feature is not apparent, the faintly defined cavity lies distinctly on the right side. Far forward, near the extremity of the plate, there is again a faint indication of a small cavity. One peculiar feature of the plate is the presence in or between the cells

(one can not in this case say which) of numerous rather large, rounded, intensely staining granules, very similar to those described by Bonnet (1901) in the completion plate of the dog. Although a few of these granules can be seen in other locations, as also figured by Bonnet, they are by far most numerous and conspicuous in the completion plate.

The variations in size of the head process and completion plate, especially as regard their breadth and the roughly corresponding variations in the lumen, are shown in figure 4. Although such variations are recorded (*cf.* Rabl, *l. c.*, Taf. iv), they are unusually distinct and regular in this case. What significance may attach to them we can not say. They seem too small to correspond with the future segmentation of the mesoderm lateral to them, and we have been unable to discover any special features in this mesoderm, such as possibly more active proliferation of cells in relation to the enlargements, either opposite or between them.

The mesoderm in the anterior half of the blastoderm is essentially the same as that which we have already described; far anterior it becomes very thin.

Any indications of a folding off of the embryo, of a proamnion, or bucco-pharyngeal membrane are wanting.

The structures thus far considered comprise the essential features of the embryonic anlage. Nowhere, as far as we can make out, is there any sign of future segmentation, and nowhere in the embryo are there either blood-vessels or blood-cells; but at the very anterior end of the embryonic disk there occur a number of prolongations of the exocoelom under the embryonic ectoderm. These exocoelomic diverticula have a very small, distinct, but quite irregular lumen lined by cells similar to those on the neighboring yolk-sac and amnion. They appear as rather long, irregular, tubular ingrowths which take their origin from the exocoelom at the point where the mesoderm of the amnion and yolk-sac meets the embryonic mesoderm. The two anterior diverticula arise in the shallow groove under the anterior edge of the blastoderm. Of these ingrowths there can be made out about four, two on either side. The anterior pair, longer and more distinct, reach nearly to the middle line. Of the posterior pair the right is very short, while the left runs parallel to and just within the margin of the blastoderm. Judging from their location, they might stand in some relation to the future pericardial coelom.

THE YOLK-SAC.

Only approximate dimensions can be given here on account of the folding, partial collapse, and a somewhat extensive tear near the anterior end of the sac. We may estimate its antero-posterior measurement at about 2.5 mm., in a dorso-ventral line at about 2 mm., and a little less than this latter figure from side to side. As seen in the illustrations (fig. 3; plate 1, fig. 3; plate 4, figs. 1 and 2) it is still very large as compared with the embryo projecting well beyond it on all sides, particularly in front and on the right. Originally it was doubtless quite regular in shape. The surface of the sac is for the most part quite smooth and regular, but over a certain area on the fundus anteriorly it has the characteristic uneven, nodular appearance arising from the early blood formation in this region.

The epithelium lining of the umbilical vesicle varies considerably in different parts. In the axis of the embryo the entoderm consists of flattened cells which form a distinct layer except in the region of the primitive node, head process, and completion plate. Elsewhere in the embryo the entoderm is a definite layer of flattened elements whose nuclei stain less deeply and are possibly a trifle larger than those of the mesoderm just above. In the immediate vicinity of the embryo the walls of the yolk-sac consist of two thin layers of cells, usually closely applied to each other, particularly toward the anterior end, but between the layers occur scattered mesoderm cells which are much more numerous in the posterior part of the sac. Farther from the embryo the entoderm cells gradually become thicker, their cell-bodies become more definite, and they take the stain more readily. Over the fundus the lining cells are in general cubical, with well-marked boundaries. Here there is extensive formation of blood-cells and blood-vessels which we shall not discuss at present, except to say that there is no connection between these vessels and those of the body-stalk. In the fundus of the sac there occur two small outpocketings of the entoderm into the covering mesoderm (fig. 3). In these diverticula the epithelium is higher and its cells larger than elsewhere.

THE ALLANTOIS.

The allantois is given off from the yolk-sac a short distance behind the cloacal membrane. It immediately enters the body-stalk, running at about right angles to the plane of the embryo and, as noted on page 118, is at one point in intimate contact with the amnion. Its length, without reference to its slightly curved course, is about 0.65 mm. The lumen is largest just above its origin in a small funnel-shaped depression in the yolk-sac. Its free, slightly coiled extremity has a cavity almost as large as at its origin, while between these the duct and its lumen are narrowest. The average outside diameter is about 0.04 mm. Its walls are composed of low columnar cells containing large, densely-staining nuclei. As it appears in the sections the duct lies in a large space due to the shrinking away of the surrounding tissue.

THE BODY-STALK.

The body-stalk is short and distinctly flattened from side to side. Embedded in the loose mesenchyme of which it is composed are the allantois, the posterior part of the amnion, and numerous vessels filled with nucleated blood-cells. No attempt has been made to reconstruct or learn the exact disposition of these channels. In places their walls seem to be deficient and they take on the character of the unlined spaces. The outer covering of the body-stalk is a rather prominent mesothelium, which is best marked near the embryo and also on what we may call the posterior aspect of the stalk. Toward the chorion this covering stops abruptly, at a varying but short distance from the attachment of the stalk, and there also appear to be deficiencies in this covering, especially on the anterior surface of the stalk. From this mesothelial layer there are a number of more or less definite ingrowths, a few of them forming quite distinct "funnels." Other findings in the body-stalk are the unlined spaces, angiocyts, and angioblast cords described by Bremer (1914).

Connections between these last-named structures and the mesothelial ingrowths are not especially in evidence, but we have not gone into a detailed study of them in this respect. The occurrence of similar conditions in the wall of the yolk-sac we would not like to exclude, because the histological pictures are here less satisfactory than anywhere else in the whole specimen.

III. THE CHORION (MICROSCOPIC) AND EXOCÆLOM.

Examination of the sections (plate 1, figs. 3, 4, and 5) shows that the villi in the equatorial zone are much more developed, longer, larger, more numerous, and more richly branched than on the two flattened poles of the vesicle. The mesodermic portion of the chorionic wall is a thin, fairly uniform stratum, the ragged exocœlomic surface of which is in marked contrast with the same surfaces of the embryonic structures. Within the larger villi, even far from the embryo, are found numbers of open spaces, some of the smaller having a fairly distinct endothelial lining. Some of the unlined spaces in the wall of the sac are very large, and they may be brought about in part or at least accentuated by the pulling away of the mesodermic from epithelial constituents of the chorion which is in evidence almost everywhere. Occasionally short strands resembling the angioblast cords are seen, even in the bases of the villi, but these and undoubted vessels in the villi are by far most frequent near the attachment of the body-stalk.

The villi possess a loose mesenchymal core which in the shorter ones extends quite to their free ends, while in the longer and larger equatorial villi this core is not so extensive. The inner layer of the epithelial covering of the villi and also of the chorionic wall is made up of distinct cellular elements, polygonal in outline and varying from thick squamous to low cuboidal, constituting the cytotrophoblast on the layer of Langhans. Cell-boundaries are here uniformly distinct, and both the cytoplasm and the nuclei stain more lightly than the same parts of the overlying syncytium. Often the line between the Langhans layer and syncytium is very sharp, again decidedly vague, while in numerous places either layer may be so reduced as to seem the only covering of the mesodermic core. Most frequently it is the syncytial layer which is so markedly thinned or apparently absent. The fact that the line between these two layers can not always be seen, and the occurrence in the deeper portions of the syncytium of what seem to be indistinct cell-boundaries, would point to the close genetic relationship of the two layers. Distally the cellular layer of the villi passes over into the cell-columns by means of which the villi are extensively united. This is especially conspicuous in the case of the equatorial villi, among which are also found extensive irregular masses, the trophoblastic cell-islands, which on the surface toward the ovum gradually merge into the cell-columns of the villi. These cell-islands are composed of large, very pale cells with distinct boundaries and large, pale nuclei. The constituent elements are for the most part irregularly polygonal, but they may take on an elongated, spindle-like form, as if actively drawn out. A faint vacuolization is not infrequent. In many places, but most marked in the neighborhood of the embryonic attachment, these cellular masses form practically an inclosing shell over the intervillous spaces beneath.

The syncytium, or plasmoditrophoblast, over the vesicle wall and the bases of the villi consists of a thin layer of slightly varying thickness, but as a rule thinner than the cellular layer beneath it. Both its cytoplasm and nuclei stain very densely. Traced outward upon the villi, the syncytium rapidly thins out on the cell-columns and soon disappears. The largest syncytial masses are found in the equatorial zone just outside the cell-islands. Here it forms large, often extensively vacuolated or spongy masses which can not always be definitely separated from the cell-islands. Scattered through the intervillous spaces, some of them close to the wall of the vesicle, are free syncytial masses of every possible size and shape. The nuclei vary widely in number; they may be small and stain quite densely, or large and pale, and this in the same bit of syncytium. "Prickle processes" are seen quite distinctly on some of these masses and their protoplasm is often very finely vacuolated. Smaller fragments of syncytium often lie in shallow pits or excavations in the cell-islands or trophoblastic columns. These masses are often very small, with one or more nuclei, and are only very lightly stained. Here again there seems to be a direct transformation of cytotrophoblast into plasmoditrophoblast. If there are evidences of cell-division in the chorion they have so far eluded us.

The amount of maternal blood in the intervillous spaces varies considerably in different localities. In a few places it is very abundant, in others almost wanting. It is most plentiful on the flattened poles of the ovum, where the villi are fewer and shorter and where the cell columns and islands and syncytial masses are least in evidence. It would appear as if the anastomosing cell-columns around the equator of the ovum had prevented the entrance of maternal blood, except very indirectly through the more distant intervillous spaces. That the blood should have drained out more readily from these deeper spaces, many of which are closed externally by the remains of the trophoblastic shell, seems quite improbable. Over much of the ovum externally is a layer of clotted blood in which leucocytes are more numerous than in the blood in the intervillous spaces.

In concluding this account of the chorion mention may be made of a small cyst-like structure faintly seen on plate 1, figure 3. It is composed of tissue to all appearances like the mesoderm of the chorion and lies close to, but seemingly not in connection with, the vesicle wall. No indications of a chorionic duct have been encountered.

Concerning the magma in the exocoelom, it will be recalled that upon gross examination of the ovum a few fine strands were observed connecting the yolk-sac and chorion. At that time it could be seen that traction upon these strands was not without effect upon yolk-sac. In the sections there can be found only some ragged wisps of a finely fibrillar nature, which at various points grade insensibly into irregular clumps of a finely granular or fibrous character extensively present in the cavity of the vesicle. In a few places where the larger strands have an attachment to the chorion there occur very intensely staining nuclei. Where best developed the fibrils are very conspicuous; they form loose bundles and stain very dark with hematoxylin. Over the amnion and the yolk-sac near it is a very thick, condensed layer of a finely granular texture (plate 2, figs. 5 and 6).

IV. GENERAL DISCUSSION.

The embryo which we have just described represents an extremely interesting and instructive stage in the ontogenesis of man. In it are found as many important features of early development as could well be expected in one and the same specimen. Besides presenting so many typical and classical features, it has the added advantage of showing them on an unusually large scale. This size, as already mentioned, may be considered simply as a variation; accentuated it may be by unknown influences. It is well known that certain developmental stages are quite ephemeral; that there is further a greatly varying susceptibility in different tissues and in these at different times, and herein may lie some explanation of the conditions described above, perhaps an unusual development or late persistence from unknown causes. We may recall here that Rabl makes repeated mention of considerable variations in size, age, and development in the *area embryonales* of rabbits, often insisting that they can not be looked upon as either abnormal or distorted, although offering no explanations. We may quote in this connection his own words (*l. c.*, p. 378; *cf.* also Taf. iv) regarding embryos with one somite: "Da habe ich denn von einer sehr merkwürdigen Erscheinung zu berichten. Ich habe nämlich zwei Arten von Embryonen dieser Entwicklungsstufe beobachtet: die eine war kurz, breit und gedrunken, die andere lang, schmal und schlank." The gist of the above is that we consider our embryo normal, though not typical.

Any discussion of the findings in this embryo naturally revolves around the question of gastrulation and the formation of the germ-layers. We shall not at this time attempt an extended treatment of the subject, but give simply our own interpretation of what we have observed in this particular case. Naturally one should not conclude too much from a single stage, either as to antecedent or later conditions; but every stage must be in harmony with those which precede or follow, and the truth is not always commensurate with the extensiveness of the evidence. On many problems of development this embryo of course throws no light whatsoever, being far too advanced.

As regards the formation of the amniotic cavity and the yolk-sac, we may accept them as currently given. The question of the mesoderm is not so easily disposed of. In spite of its precocious development, we can not yet see the necessity of denying that it may still be, in principle, peristomal mesoderm. Considering the recent attempts of Rabl in this respect and the similar difficulty regarding the entoderm, it would seem to us that the inherent questions of gastrulation and homology should be more definitely disproven before an entirely new and foreign mode of development is invoked.

In the primitive streak we have a closed blastopore, howbeit radically altered. At its anterior end is an opening and what is theoretically at least an invagination, the head process with its archenteric canal. The posterior end of the streak is, in this stage, marked by the cloacal membrane which is later also open, at present in process of formation. Between the two points there is extensive mesoderm formation, as witnessed by the mitoses in this region, peristomal mesoderm. If one were

inclined to carry the comparison still farther, the peculiar features of the primitive groove mentioned on page 121 might be interpreted as an attempt at the formation of lateral blastoporic lips. How much of the mesoderm of the embryo appears first as strictly peristomal we of course can not say.

The consideration of the head process involves also the tangled question of the entoderm. The head process of the primitive streak (Kölliker), l'ébauche de l'archentéron of Van Beneden, Bonnet's Urdarmstrang, or the Mesodermsäckchen of O. Hertwig, is one of the most important features of the area embryonalis. In its formation, and that of the primitive streak, we have the essentials of gastrulation in man; in the cavity of the head process, the archenteric canal (Urdarmkanal), is retained all that is left of the cavity of the primitive gastrula, the archenteron. From this head process are derived, to what extent it is impossible to say, gastral mesoderm, further chorda, for the most part, and (for aught we know) more or less of the entoderm of the gut-tract. From the foregoing it will be clear that we do not agree with Keibel (1910, 1913) and Hubrecht (1905, 1909) in considering the formation of the two-layered stage, ectoderm and entoderm, as constituting the process of gastrulation. That entoderm formed by delamination is essentially secondary or yolk entoderm, the paraderm of von Kupffer, Wenekebach's *cænogenetic* entoderm, the *lécithophor* of van Beneden.

To what extent this first-formed layer is concerned in the formation of the digestive tract we do not know; certainly in some forms its rôle is by no means an exclusive one. The fact that this yolk entoderm fuses with the head process but not with the primitive streak is but evidence as to its *cænogenetic* character. The only support of the views of Keibel and Hubrecht is the supposition that this secondary entoderm is the sole and only source of the gut entoderm. The theory and the entoderm stand or fall together. In the walls of the head process, *i. e.* bounding the archenteric canal, we would expect to find primary or protentoderm, Bonnet's Urentoderm, the *palingenetic* entoderm of Wenekebach. If the lumen of the head process is in reality an archenteric canal, then we would expect it to give rise to mesoderm (segmented), chorda, and gut entoderm—and such, with the reservations given above, seems to be the case. If the head process is simply the anlage of the chorda plus some mesoderm (whence the misnomers chordal or notochordal canal, chordulation, etc.), why should it contain a definite although inconstant canal communicating with the exterior; why so much more material than is required for the chorda, and why its fusion and communication with the yolk-sac? The answer is that in the formation of the head process and not in the delamination of the secondary entoderm we have a process which can be designated as gastrulation.

As concerns the derivatives of the head process, the case of the chorda is perfectly clear. At this stage its anlage is contained in the dorsal, epithelial wall of the canal, the notochordal plate. The fate of the ventral wall or floor of the canal, the plaque *entérique* of van Beneden, is uncertain. It fuses early with the yolk entoderm or *lécithophor* immediately beneath to form the plaque *lécithoentérique*. The loss of the floor, from the rearrangement of its cells, results in the confluence of the archenteric canal and the cavity of the yolk-sac. This process is naturally *cænogenetic*,

since the yolk entoderm and its inclosed cavity are cænogenetic features. There are thus restored the original conditions in which the anlage of the chorda and mesoderm (enterocœle) are situated in the dorsal wall of the gut. To what extent there is any formation of gastral mesoderm from the head process is a question. In any case, even if the mesoderm had a peristomal origin, its continuity with the walls of the canal is sufficient to indicate the interpretation of the latter as potential sources of gastral mesoderm. With the disappearance of the floor of the canal there is ushered in the stage of the so-called intercalation of the chorda in the entoderm. This obviously takes place quite irregularly and the picture is exactly that seen on such a large scale in Reptilia, but clearly marked in many other forms. This stage is shown on plate 2, figure 4, and plate 3, figure 4. To be exact, this is not an intercalation of the chorda in the entoderm. The notochordal plate is in connection laterally not only with the entoderm, but much more extensively with the mesoderm. If one suppose that there may still be mesoderm formed from the borders of the plate (and there is here no evidence to the contrary) it would be possible to raise objection to the use of the term "notochordal plate," since it would contain chorda and gastral mesoderm. Not, however, until there is a definite separation of the plate from the mesoderm and its continuity with the entoderm alone can one speak of an intercalation of the chorda.

The extent, if any, to which the plaque lécitoentérique (Dotterdarmplatte) contributes to the formation of the wall of the future digestive tract is difficult to determine and certainly not to be decided by any one stage. There are a number of facts, however, which seem to point to such a participation. The marked disproportion between the notochordal and enteric plates in the posterior, least differentiated part of the head process and the retention of the former, practically intact throughout its whole extent, indicate unmistakably that there is formed from the primitive node and head process a considerable mass of material which is not expended in the formation of the chorda. If this material be not actually lost, then it must find its way into the mesoderm or entoderm or into both. In view of the large mass of material produced, much greater than that destined for the chorda, and considering also its peculiar mode of development, virtually an invagination, the simplest solution is to suppose that both mesoderm and entoderm are formed from the side-wall and floor of the head process. If the development of gastral mesoderm is small or wanting, so much more material for the entoderm. It may be recalled here that the digestive tract in the embryo is very small below the pharynx and no very great amount of material would be required to form its walls. The fact that in certain animals the primary entoderm is concerned in the formation of the epithelial wall of the gut seems to us very significant. It would seem that the absence of definite evidence that the entoderm of the future embryo is not, in part at least, primary entoderm, is outweighed by the above considerations and by the fundamental homologies which they tend to preserve.

With the head process and primitive streak we have not yet exhausted the possibilities for the discussion of fundamental problems; there remains the question of the completion plate, Bonnet's *Ergänzungsplatte*, the protochordal plate of

Hubrecht. Our knowledge of this structure is less extensive than that of the region we have just been considering, and our remarks will be correspondingly brief. Bonnet's term is a very fitting one, since the derivatives of the plate are the same as those of the head process and directly continuous with them. For Rabl (*l. c.*, p. 239) the completion plate is simply "das vorderste Ende des in Lecithophor vorgeschobenen Kopffortsatzes oder Urdarmsäckchens." For Bonnet and Hubrecht it is developed from the yolk entoderm independently of the head process. The evidence in this particular case would seem rather to support this latter view. The second view, however, is not so easily reconciled with our ideas of gastrulation as the first, and we shall not carry the discussion farther at this time. Concerning the future of this plate, which has been recognized in a variety of forms and given a variety of names, there is much more unanimity of opinion. There arise from the completion plate in the dog, according to Bonnet (*l. c.*, p. 286): "1. Mesoderm des Vorderkopfes, 2. die Chorda des Vorderkopfes, und 3. ein prämandibuläres Darmrudiment. Es bildet dieses Gebiet also thatsächlich ein Ergänzungsstück des Urdarmes, indem es dieselben Derivate wie dieser aus sich hervorgehen lässt." The anterior part of the chorda, which, as compared with that derived from the head process is very short, remains long in connection with the entoderm. The formation of mesoderm is also continued here for some time.

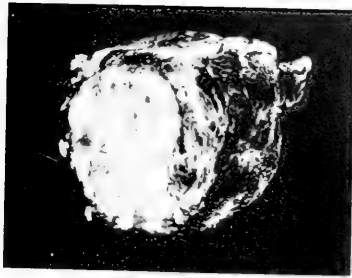
The significance of the apparent cavity formation in the completion plate is a matter of uncertainty. It might be compared with the secondary canals which sometimes appear in the chorda as it separates from the entoderm. One could perhaps look upon them as attempts at the development of an archenteric cavity, or they might conceivably stand in some relation to the rarely appearing head cavities. As far as we can make out, the buccopharyngeal membrane would have appeared close to the anterior limit of the completion plate, with the possibility of the plate contributing in its formation.

From the observations here presented and from the consideration of other human embryos, one may conclude that the essential features of gastrulation in man are directly comparable with the classical features of that ancient and important process. Significant parallels may be drawn between early human ontogenesis and that of many other representative vertebrates. The conditions in man are manifestly simpler and more primitive than in many cases which have been extensively studied, these being often very specialized or aberrant forms. Hand in hand with specialization and advancement there is the appearance in ontogeny of cænogenetic features which always tend to obscure the original picture. If man has retained much that is primitive and generalized, then we should expect to find some expression of this in his earliest development.

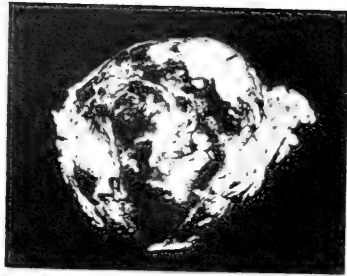
CLEVELAND, OHIO, September 27, 1917.

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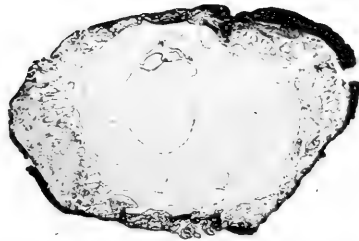
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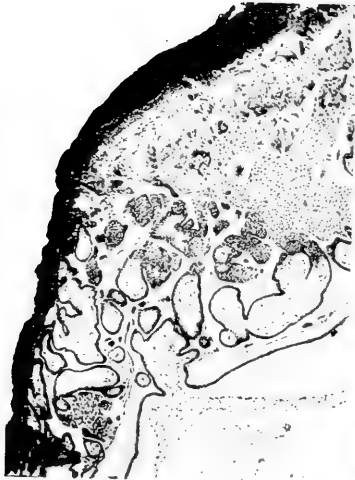
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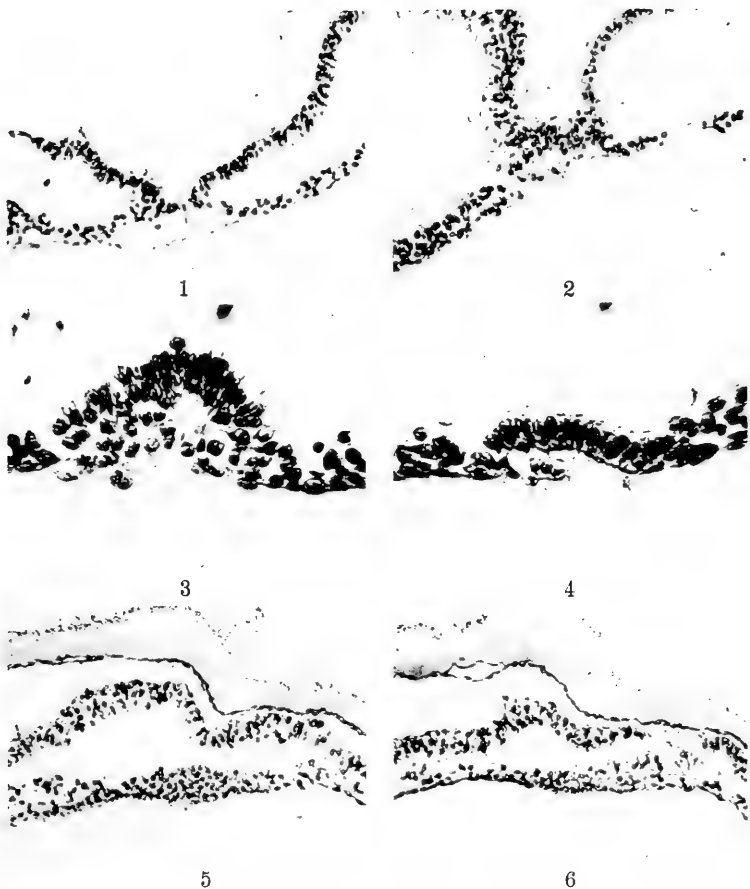
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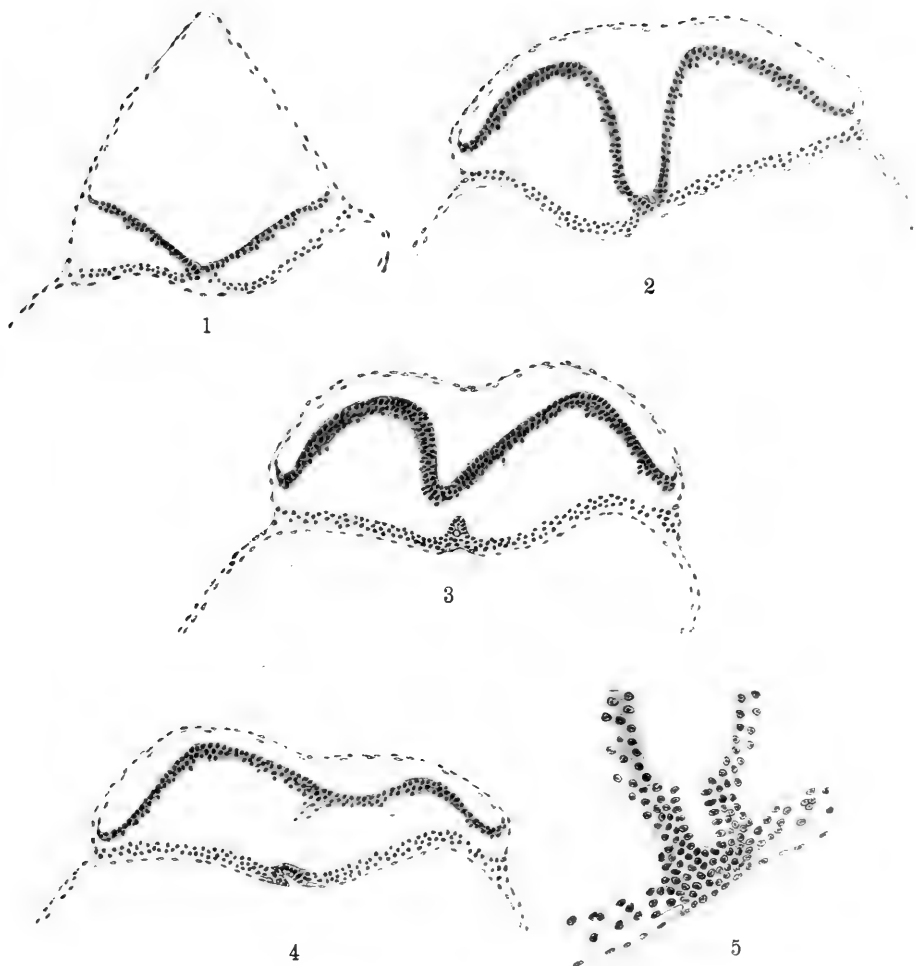
1 and 2. Intact vesicle, opposite views. $\times 4\frac{1}{2}$.
 4. Detail of Fig. 3 (lower left corner). $\times 45$.

3. Photograph of Section 404. $\times 9$.
 5. Detail of Fig. 4. $\times 180$.



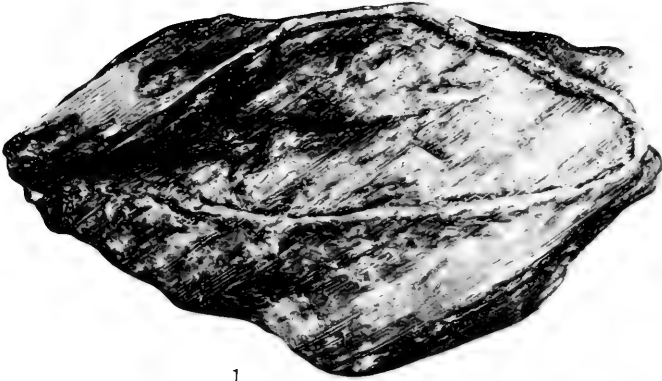
Photographs of sections of axial structures, the location and direction of which are indicated in the text-figures. In all the photographs and drawings the right side of the embryo is on the left in the plates, i. e. all views looking caudad. Dorsal structures are cut slightly more anterior than ventral ones. (Cf. text-figure 3.)

1. Section 425. Posterior part of primitive streak. $\times 160$.
2. Section 406. Most caudal section in which archenteric canal appears. Its location may be recognized by the absence of nuclei. $\times 160$.
3. Section 401. Archenteric canal where largest and best defined. $\times 400$.
4. Section 395. Large (second) ventral opening, "plaque notochordale" very distinct. $\times 400$.
5. Section 380. Completion plate where best developed. $\times 160$.
6. Section 375. Completion plate near anterior limit. $\times 160$.

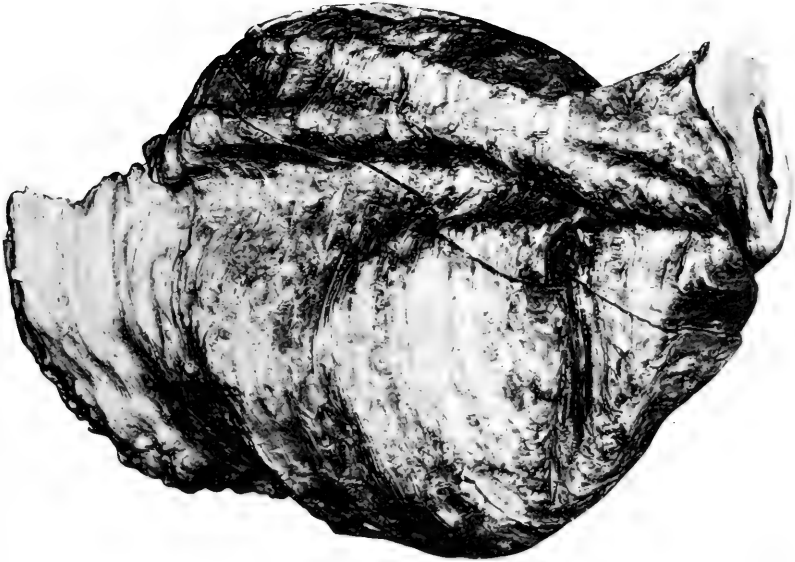


1 to 5. The following drawings (indicated by D in the text-figures) are obviously reconstructions and therefore somewhat schematic. They would cut the median line at or near the points traversed by the sections shown in the photographs (Plate 2, figs. 1 to 4), and have therefore been designated by the same section number. In other words section (photograph) 425 and drawing (section) 425 would intersect in the median line. Like the photographs they are slightly oblique dorsoventrally.

1. (Section) 425. Posterior part of primitive streak. $\times 100$.
2. (Section) 406+. Dorsal opening of archenteric canal. $\times 100$.
3. (Section) 401. Typical head process, archenteric canal, "plaque notochordale" and "plaque lécithotérique." $\times 100$.
4. (Section) 394+. Large ventral opening, "plaque notochordale." $\times 100$.
5. Idealized cross-section (reconstruction) at the point where all three germ-layers are continuous. Its location, while not indicated, can be made out from text-figure 5.



1



2

1. Dorsal and slightly lateral view of model. $\times 100$. Reduced. On the left the body-stalk is cut across.
2. Left lateral view of model. $\times 100$. Reduced. On the right the body-stalk, cut in the plane of the sections, shows a large vessel; running downward and forward, also in the plane of the sections, a small portion of the amnion has been left. In the embryonic disc are seen the irregular ectodermic folds, its anterior extremity is undermined. The irregularity in the anterior part of the yolk-sac is due to a tear. The upper part of the model has been separated from the lower by sawing through it parallel with the blastoderm; it is this upper piece which is represented in Fig. 1.

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CONTRIBUTIONS TO EMBRYOLOGY, No. 24.

**THE DEVELOPMENTAL ALTERATIONS IN THE VASCULAR SYSTEM
OF THE BRAIN OF THE HUMAN EMBRYO.**

BY GEORGE L. STREETER.

With five plates and twelve text-figures.

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THE DEVELOPMENTAL ALTERATIONS IN THE VASCULAR SYSTEM OF THE BRAIN OF THE HUMAN EMBRYO.

BY GEORGE L. STREETER.

INTRODUCTION.

One of the most striking features in the development of the blood-vessels of the head is the clear way in which they demonstrate the embryological principle of what may be termed integrative development. It is quite evident that the vascular apparatus does not independently and by itself "unfold" into the adult pattern. On the contrary, it reacts continuously in a most sensitive way to the factors of its environment, the pattern in the adult being the result of the sum of the environmental influences that have played upon it throughout the embryonic period. We thus find that this apparatus is continuously adequate and complete for the structures as they exist at any particular stage; as the environmental structures progressively change, the vascular apparatus also changes and thereby is always adapted to the newer conditions. Furthermore, there are no apparent ulterior preparations at any time for the supply and drainage of other structures which have not yet made their appearance. For each stage it is an efficient and complete going-mechanism, apparently uninfluenced by the nature of its subsequent morphology.

With these factors in mind, one can better understand the architectural arrangements of the vascular system of the head that appear in different periods of development. In the primordial or precirculatory period the vessels that then exist are engaged principally in growth and in the elaboration of a plexus, and their form then is little influenced by conditions that would favor the circulation of the contained fluid. As the circulatory flow of the blood becomes established we find that the vascular plexus responds by conforming to the hydrodynamic requirements, becoming adequately adapted to the form of the neural tube as then existing, with favorably situated aortic feeders and simple and direct drainage channels. As the brain becomes more complicated, and as the skull-membranes form, there occur, step by step, the necessary adaptations on the part of the blood-vessels. Finally, when the permanent form is attained, the vessels lose their transitory character and develop permanent and more highly differentiated walls, properly suited to the adult functional requirements.

It is possible to subdivide the development of the blood-vessels of the brain into five successive periods, each showing special adaptations to their changing environmental conditions. To facilitate the description of this process an arbitrary order of that kind will be adopted in this paper. During the first of these five periods there are established the primordial endothelial blood-containing channels, which are neither arteries nor veins, but constitute the source from which all the arteries, veins, and capillaries of the brain are derived. These primordial blood-

vessels lie bilaterally close along the brain-wall, at first either in the form of a single, slender, longitudinal tube, as seen along the hindbrain, or a plexiform space as seen near the forebrain and midbrain. Soon after the primordial vessels are established, endothelial buds sprout from their walls and in conjunction with them form an irregular endothelial vascular meshwork which tends to spread over the surface of the brain-wall, especially in the region of the forebrain and midbrain. There is thus formed a plexiform system which constitutes a germinal bed of endothelium rather than a circulatory apparatus.

During the second developmental period the primordial blood-vessel plexus of the head slowly resolves itself into veins, arteries, and capillaries, and becomes architecturally suited to the circulatory flow of the blood. That portion of the plexus which lies against the brain spreads out as a flattened capillary sheet, conforming everywhere to the shape of the brain-wall and its attached ganglia and sense-organs. The more superficial part of the plexus develops a coarser mesh and forms larger channels, which tend to unite into continuous trunks and gradually, by virtue of their communications, can be recognized as definite arteries and veins. The intermediate loops of the plexus maintain the anastomosis between the deep capillary sheet and the more superficial trunks, forming tributaries of the veins and branches of the arteries. The second period thus establishes the primary type of the circulation of the head, in which there is a capillary bed, fed by arterial branches from the aortic system and drained bilaterally by a continuous venous trunk which extends back to the venous end of the heart.

The third period is inaugurated by the differentiation of the membranous skull, the dura mater, and the arachnoid-pial membranes. As a result of this stratification of the tissues of the head, the more ventral of the anastomosing channels that connect the deep capillary plexus with the superficial vessels become closed off and there is a general separation or cleavage of the vessels immediately surrounding the brain-wall from those belonging to the membranous skull and its coverings. This process begins at the base of the skull and extends bilaterally upward toward the middle line of the vault, in which region the communications between the deeper and more superficial systems are to some extent maintained. In this way the cerebral vessels are gradually separated from the dural vessels and in a similar manner the superficial vessels of the head become isolated by the laying down of the primordium of the membranous skull, after which their course of development is quite independent of that of the dural and cerebral systems.

By the time the third period is well under way it is overlapped by the fourth period, under which we include the remarkable series of adjustments in the arrangement of the blood-vessels, in adaptation to the developmental alterations in the form, size, and condition of the structures of the head region. The brain is one of the chief factors in this process. The marked change in its form, and especially the prolonged relative growth of the cerebral hemispheres, render necessary a continuous series of alterations in the blood-channels that extend far into the late fetal stages. In the earlier stages a fundamental change results from the growth of the labyrinth and its cartilaginous capsule, whereby a mechanical obstruction is

introduced that results in the obliteration of a considerable part of the largest vein of the head. This is compensated for by a new channel which takes a more dorsal course, and which eventually forms the sigmoid portion of the lateral sinus.

Finally, under the fifth period we would include the late histological changes in the walls of the vessels that convert them into the adult arteries, veins, and the various types of sinuses. These histological factors, however, are not considered in the present paper and are merely mentioned to complete the sequence, as they have no determining influence on the phenomena of the preceding four periods.

The observations that are reported are chiefly concerned with the third and fourth developmental periods; that is, after the primary circulation of the head is established and during the cleavage and adjustmental stages. A review, however, will be made of the history of the vascular system of the head previous to that time, which will be based in large part on the important observations of Evans (1909, 1912) and Sabin (1915, 1917*a*, 1917*b*). The study of the development of the vascular system of the head of the human embryo was initiated in this laboratory by Professor Mall (1905). Later I continued the same investigation and reported (Streeter, 1915) some of the features of the adaptive metamorphosis of the dural veins. In the present paper I shall include much of the same subject-matter and incorporate with it further observations made on additional material, thus making it possible to treat the general subject more completely and to demonstrate the relations of the dural veins to the principal arteries of the head.

ESTABLISHMENT OF PRIMORDIAL VASCULAR SYSTEM OF HEAD.

A distinct advance in our knowledge of the origin of the vascular system has recently been made by Professor Sabin through the study of living preparations of the growing chick. By that means it was possible to observe directly the principal steps in blood-vessel formation. Certain steps in the process that were still under dispute have thus become established, as well as others which are new observations. According to her description (Sabin, 1917*b*), the vaso-formative cells or angioblasts are differentiated from the mesoderm, and on proliferation they form small, dense, syncytial masses which join one another by means of tiny processes of cytoplasm. In this way there are formed plexuses of solid angioblastic cords, the growth of which is maintained partly by proliferation of their constituent cells and partly by the further addition of new angioblasts which differentiate from the adjacent mesoderm.

During the formation of these angioblastic plexuses a liquefaction of their cytoplasm occurs in such a manner as to convert the solid angioblastic cords into vessels filled with clear fluid. This is brought about by a process of vacuolization and the formation of an "intracellular" lumen. Vacuoles appear near the nuclei and rapidly enlarge, so that in from one to two hours there occurs a complete destruction of the cytoplasm and nuclei of the central part of the angioblastic cords, resulting in the formation of a clear plasma, the periphery of the angioblastic mass being preserved as an endothelial boundary. The liquefaction of cytoplasm occurs both in the loops of the angioblastic plexuses and in masses of angioblasts

that are still isolated, in the latter case resulting in small vesicles that subsequently join the main plexus. These observations were made for the most part in the area pellucida of the yolk-sac, but the same phenomena were seen also within the body of the embryo. The dorsal aorta in the trunk region and a portion of it within the head was seen to differentiate *in situ* in the above manner.

The angioblasts are not all converted into endothelium and blood-plasma; some of them take part in the formation of red blood-corpuseles. During the lumen formation in the angioblastic strands, small clumps of the original angioblasts become partially separated by the liquefaction of the cytoplasm around them. Such masses soon show the presence of hemoglobin and constitute blood-islands which eventually break apart as free red blood-cells and float away in the blood-plasma. Blood-islands can also be seen to be derived secondarily from the endothelium.

Concerning the distribution of the earliest blood-vessels and concerning the form and development of the primordial vascular system of the head, I have followed the descriptions published by Evans (1912) and Sabin (1917*a*), both of whom studied injected specimens of the chick and pig. On account of the difficulty of making observations of this region in living preparations, the details in the growth of this endothelial meshwork have not been actually seen. The plexiform transformation of the aortic arches has been demonstrated only partly and it is not known how much of the first endothelial system of the head is derived from angioblasts that are differentiated locally and how much to the proliferation of the cells of the aortic arches. It is possible, even after the laying-out of the main parts of the primordial system, that angioblasts continue to differentiate in the new territory around its margins and become incorporated with it. The essential features, however, in the development of this system are admirably shown by injected material, as can be seen in figures 398 (duck, 13 somites) and 393 (chick, 15 somites) of Evans (1912), and plate 1, figure 3 (chick, 9 somites), and plate 2, figure 1 (chick, 14 somites) and figure 2 (chick, 16 somites) of Sabin (1917*a*).

At about the time that the dorsal aortæ become established in the head region, it is seen that the first pair of aortic arches connecting them with the heart are plexiform in character. Sprouting from this plexiform area an endothelial meshwork arises and extends dorsalward and caudalward toward the forebrain and midbrain, to become the primary head-plexus. Very soon afterwards, a slender longitudinal channel is formed bilaterally along the ventro-lateral margins of the hindbrain which communicates with the primary head-plexus in front and caudally with the anterior cardinal vein by way of the transverse veins of the first two interspaces. A few slender communications are established very early between it and the dorsal aorta of its respective side. This channel has been designated by Sabin (1917*a*) as the "vasa primitiva rhombencephali." It seems probable from her observations that this channel is not a derivative from sprouts from the dorsal aorta or from the primary head-plexus, but is differentiated *in situ*, and its communications with them are secondary. This slender channel, together with the primary head-plexus, constitutes the primordial system from which all the blood-

vessels of the brain and its membranes are derived. Sabin has suggested a greater restriction in the use of the terms "artery" and "vein" and has warned against making a too early identification of the adult vessels in the embryo. The primordial vascular system of the brain as seen in the chick of 15 somites consists morphologically of a sprouting meshwork rather than a set of definite supply and drainage channels. It is to be considered as a bed of proliferating endothelium rather than as a circulatory apparatus. Even after the blood begins to move through its meshes it is some little time before the circulatory function becomes the dominating influence in the determination of its architectural features, and thus, in these early stages of the vascular system, we meet arrangements which, as regards their form, size, and communications, are distinctly inefficient as to the circulatory flow of the contained blood, but are quite characteristic of the germinal period of an endothelial meshwork. Any attempt to distinguish arteries from veins in this primordial system results only in hopeless confusion.

DIFFERENTIATION OF PRIMORDIAL SYSTEM INTO ARTERIES, VEINS, AND CAPILLARIES.

Our information regarding the angioblastic period in which the primordial blood-vessels are laid down has been derived mainly from study of chick embryos. The transition into the second developmental period in which the primordial system gradually undergoes resolution into arteries, veins, and capillaries has been demonstrated in mammalian material (pig embryos), and toward the end of the period, as the primary circulation becomes established, the chief features have been described in human embryos. As illustrating these transitions, the reader is referred to figures 394, 395, and 400 of Evans (1912), and figure 1, plate 4, of Sabin (1917*a*). From the time when the primordial blood-vessel system of the head is first laid down, its endothelial walls undergo active proliferation and a sprouting meshwork extends from it, invading the interval between the ectoderm and the brain-wall. This spreading of the endothelial plexus is more active in some directions than in others. In general it tends to spread toward and over the surface of the neural tube and the nerve structures connected with it. It extends early over the midbrain and forebrain and soon encircles the optic stalk. Along the hindbrain the plexus formation is somewhat slower. In fact, in the chick the primordial vessel in this region persists as a simple channel until the embryo has acquired about 29 somites, by which time the growth in the more cephalic region is quite extensive. Persisting so long unchanged, as it does in the chick, it was named the "vena capitis media," in contradistinction to the more laterally situated channel that develops later, the so-called "vena capitis lateralis." It was shown, however, by Sabin (1917*a*) that what exists here in the chick at this time is the persisting primordial channel and not a true vein.

In its earlier stages the proliferating meshwork shows considerable irregularity in the form and size of its constituent channels. Gradually it can be seen that the more superficial loops of the mesh are taking the form of larger and more directly continuous channels. The deeper loops of the mesh flatten out in a more uniform capillary sheet that lies in direct apposition to the brain-wall and its attached

structures. The intermediate portions of the mesh maintain the communications between the deep capillary sheet and the superficial main channels. The few communications of the aortic system with the intermediate and deeper part of the plexus are maintained and unite with selected loops of the plexus to form slender continuous channels which are eventually lost in the plexus. In this way the irregular plexus of proliferating endothelial tubes is resolved into a definite circulatory system, consisting of a capillary mesh that is fed by deep branches from the aortic system and drained by superficial tributaries into a main channel that extends backward to empty into the common vitello-umbilical vein. By the time that this is accomplished the contained blood is already slowly circulating through this primitive system.

The main drainage-channel which thus becomes established on each side of the head was originally called the anterior cardinal vein until it was recognized that only the caudal portion of it properly belonged to the cardinal system. Evans (1912, p. 676) suggested, as more appropriate for it, the name "primitive head-vein," and the same term was utilized by Sabin (1917*a*). This primary head-vein develops essentially in the same manner both in the chick and the pig—that is, through the elaboration of a simpler and more direct channel through the superficial part of the proliferating plexus.

The difference, however, in the anatomy of chick and pig embryos is associated with some difference in the details of the development of this primary head-vein. The pre-trigeminal portion of it is identical in both forms and consists of a superficial main channel, receiving tributaries from the deeper part of the plexus. It forms in the interval between the thalamus and the optic bulb and leads backward, median and ventral, to the trigeminal ganglion, where it temporarily connects with the primordial system. The middle portion of the primary head-vein, the portion in the interval between the trigeminal and vagus nerves, is formed later than the pre-trigeminal portion, and is formed relatively later in the chick than in the pig. In the chick, it has been shown by Sabin (1917*a*) that the primordial channel running along the ventro-lateral margin of the hindbrain persists, in embryos of 29 somites, as a single large channel communicating in front with the elaborate plexus of the midbrain region and the already formed pre-trigeminal portion of the main drainage channel. Caudally it communicates through the transverse vein of the first interspace with the anterior cardinal vein, the latter forming the caudal portion of the primary head-vein. Thus, in the chick the hindbrain portion of the primordial blood-channel (the vasa primitiva rhombencephali of Sabin) serves temporarily as a part of the circulatory apparatus before its proliferative function has been completed, but in the stage to which we are referring proliferating loops have begun to spread from the main channel over the wall of the hindbrain and its attached ganglia. Derived partly from these and partly from the plexuses of the gill-arches, there is formed a series of superficial loops which link themselves together into a slender longitudinal channel communicating in front with the pre-trigeminal portion of the main drainage-channel and extending backward lateral to the otic vesicle, to empty into the anterior cardinal vein. This consti-

tutes the middle portion and completes the formation of the main drainage-channel of the head, the primary head-vein. In the pig, the primordial blood-channel along the margin of the hindbrain, instead of enlarging as a simple temporary channel as seen in the chick, becomes resolved almost at once into a proliferating plexus, and from its superficial loops, and perhaps also from some of the loops of the adjacent branchial plexuses, there is evolved the middle portion of the primary head-vein, which is completed nearly as soon as the pre-trigeminal portion and before there is any considerable circulation of the blood. The caudal part of the primary head-vein is made up of the anterior cardinal vein, which, as we have seen, is originally continuous with the primordial channel, joining it (in the pig) in front of the occipital myotomes instead of through the transverse vein of the first interspace, as seen in the chick. As the more superficial loops, sprouting from the primordial system, become established lateral to the otocyst in the formation of the middle portion of the primary head-vein, their communication with the anterior cardinal becomes larger, whereas the original communication of the anterior cardinal with the primordial channel becomes more restricted and breaks up into a plexus; thus, by the linking-up of these three parts, a continuous superficial channel is formed which extends the whole length of the head and provides for the adequate and efficient drainage of all its structures.

It has been noted that, from the beginning, communications exist between the aortic system and the more dorsally placed primordial vascular system of the head. The largest and most constant are the paired trunks that connect the first aortic arches with the deeper loops of the ventral part of the forebrain plexus. What was originally a plexiform communication is later resolved into a single trunk that eventually forms part of the internal carotid artery of its respective side. Along the hindbrain region are other irregularly placed, slender communications, and on reaching the myotome region there are the segmental dorsal branches of the dorsal aortæ, which anastomose with the proliferating plexus of the neural tube. The primordial blood-channel along each side of the hindbrain proliferates in the form of a plexus, and as the plexuses of the two sides spread on the surface of the brain-wall they gradually establish an anastomosis along the mid-ventral line. At the same time a series of the more superficial loops of the plexus, in association with the communications from the dorsal aortæ, become elaborated into a slender bilateral longitudinal channel which is continued caudally into the spinal region, and orally it connects with similar loops which are associated with the embryonic internal carotid artery. There is thus established, on each side along the ventral surface of the neural tube, a continuous arterial channel which is connected dorsally by many loops with the neural capillary plexus and ventrally by a few branches with the aortic system. From these simple channels are derived later the main arteries of the brain and spinal cord.

With the establishment of the primary head-vein, we may regard the first type of circulation of the head as completed. It consists essentially of a series of arterial feeders from the aortic system, which lose themselves in the sheet of capillaries that invests the neural tube, which capillaries in turn are drained by many

anastomosing loops into the more superficially placed *primary head-vein*, and thereby are connected with the duct of Cuvier and the venous end of the heart. It is this primary arrangement that exists in human embryos 4 mm. long, and this is the earliest stage that was examined in connection with the present study. In figures 22 and 23, plate 2, are shown the left and right profiles of a model made by the Born construction method of such an embryo (Carnegie Collection, No. 588, 4 mm. long). This is slightly younger than the stage shown by Mall (1905) in his figure 3, although the conditions in the two are very similar. It is distinctly younger than both the Ingalls (1907) and the Elze (1907) specimens, although they also show the same primary type of circulation. In figures 22 and 23 the neural tube, eye-stalk, trigeminal ganglion, and ear-vesicle are shown in gray and the reconstruction was planned so as to show the relation to these structures of the main arteries and veins, the latter being colored red and blue respectively. On examining these figures it is to be remembered that only the definite supply and drainage channels are shown. In order to complete the system one must imagine the neural tube as almost completely ensheathed by a capillary sheet into which the arterial feeders open and from which the small venous tributaries arise. In figure 23 fragments of this capillary sheet showing this relation to the drainage-vessels are indicated in the hindbrain region.

The morphological details of the models will perhaps be more readily understood by the study of these two figures, in which the attempt has been made to indicate the form and relations as clearly as possible, than by the following of a descriptive text. Attention, however, may be directed to a few of the general features. In the first place, it seems to be the rule that, throughout the whole body of the embryo, the source of blood-supply has a central position and its flow to the tissue is in a peripheral direction, whereas the return drainage system lies more superficially and the flow is consequently in a central direction. As for the drainage of the head, there is provided a simple and adequate channel, the primary head-vein. Its only deflections from a perfectly direct course are those rendered necessary by the structure of the parts. Its position is affected by the trigeminal, facial, glossopharyngeal, and vagus nerves, due to their respective placodal relations to the ectoderm. It could not pass lateral to the trigeminal mass, and so is deflected inward. The faeial and glossopharyngeal ganglia lie in positions sufficiently ventral so as not to interfere with its superficial position; the former, however, deflects it upward slightly. The ganglion nodosum of the vagus lies directly in its course and, as in the case of the trigeminal, the channel is thereby forced to take a median course, since the preferred superficial course is ruled out by the placodal attachment. We thus meet with a median deflection at that point, so that the primary head-vein curves caudally around the vagus trunk.

Just as the main trunk of the primary head-vein follows the most simple and direct course possible, so its plexiform tributaries are favorably situated for draining the various areas which are present at that time. Those from the capillary sheet of the brain-tube join it for the most part on its dorsal and medial border. In accordance with the topography of the region, these tributaries are arranged in

three groups: (1) an anterior group from the forebrain and midbrain, leading into the pre-trigeminal portion of the primary head-vein; (2) a middle group from the cerebellar region, emptying into its otic or middle segment, that portion between the trigeminal and glossopharyngeal nerves; and (3) a posterior or occipital group which accompanies the vagus rootlets and empties near the junction of its middle and cardinal portions. This posterior group usually empties by a common trunk. This trunk corresponds to the original communication between the anterior cardinal vein and the primordial blood-channel of the hindbrain, which now has proliferated into the meshwork forming the capillary sheet that invests the brain-wall. Before the completion of the middle or otic portion of the primary head-vein, this trunk from the occipital group of tributaries was the only communication between the anterior cardinal and the vessels of the more oral region of the head. Especial attention is directed to these three tributary groups, as their arrangement is significant for the later stages, as will presently be seen. In addition to these tributaries from the brain, the primary head-vein receives ventral tributaries from the eye region, from the nerve-ganglion masses, and from the region of the first and second gill-arches, which communicate with the plexuses derived from the proliferating elements of the first two aortic arches.

The arterial supply to the head region at this time is primarily through the internal carotid arteries, which form relatively slender though direct channels. On each side they are made up of the trunk that connected the primary head-plexus with the first aortic arch and the portion of the dorsal aorta corresponding to the first two arches. It will be noted that the first and second vascular arches are more or less incomplete, having broken up into irregular plexuses ramifying in the tissues of their respective gill-bars. A part of the plexus of the second arch apparently becomes incorporated in the external carotid artery, although the trunk of this artery is probably represented by the short stem seen in figures 22 and 23, projecting from the oral border of the third vascular arch. The capillaries and venous drainage plexuses of the first and second arches are shown only at their point of entrance into the primary head-vein above. The internal carotid artery extends forward to the root of the optic stalk, where it bifurcates into its terminal branches, which soon become lost by anastomosis with the capillary sheet of the brain-wall. The continuation of this channel backward along the ventral wall of the brain could not be satisfactorily modeled, though this channel and its anastomosis with the basilar and vertebral arteries doubtless already exist. It is shown in the reconstruction by Ingalls (1907) in a 4.9 mm. embryo.

The cut ends of communications such as originally connected the dorsal aorta with the primordial vascular system of the brain are indicated. As long as they persist, these must be regarded as arterial feeders through the basilar arteries to the capillary sheet of the brain. One of these may doubtless be looked upon as the stapedal artery (for example, the one between the first and second arches). The vertebral arteries apparently were established in this specimen, but they could not be satisfactorily outlined and so were omitted. They must, however, be included as part of the source of blood-supply. With this manner of blood-supply,

and with this manner of drainage, as illustrated in figures 22 and 23, one must feel that the provision for the blood-circulation is well designed and is perfectly adequate for the structures as then existing, and were there no further alterations in the structures themselves no further change in their blood-vessels would be necessary. On the other hand, there are no superfluous channels present and no evident vascular provision for structures not yet developed, that is, disregarding the potential proliferative power which exists in such an endothelial system. The establishment of this primary type of the circulation of the head completes our second period in the growth of the cranial vascular system.

CLEAVAGE OF BLOOD-VESSELS OF HEAD INTO SEPARATE SYSTEMS.

The differentiation of the dura mater and the formation of the arachnoid mesh begins in the region of the base of the skull, and from there the process spreads slowly upward toward the vertex of the head. As these structures form it can be seen that the anastomosing channels connecting the capillary sheet of the brain with the more superficial drainage channels are gradually closed off, the more ventral ones first and the more dorsal ones later. In this way the dura forms a partition that results in a general separation or cleavage of the superficial vessels (consisting mainly of the primary head-vein and its tributaries) from the deeper vessels in intimate contact with the brain-wall, including the capillary sheet and the vessels supplying and draining it. The latter or deeper system, however, continues to drain into the former at certain restricted places. As this cleavage occurs, we can distinguish between dural vessels which are chiefly veins and cerebral or pial vessels which include arteries as well as veins. Soon after, coincident with the formation of the membranous skull, the dural system becomes more or less completely separated from the vessels of the integument and its subjacent soft parts. We then have three different main strata of blood-vessels—the external, the dural, and the cerebral. The most conspicuous of the early external vessels are those belonging to the integument. They make their appearance around the base of the skull in embryos between 12 mm. and 20 mm. long and spread upward toward the vault. In spreading upward they exhibit a characteristic growing edge consisting of anastomosing loops of the mesh which can be seen with the naked eye as an advancing narrow line marking off the non-vascularized area above from the vascularized part below, as has been clearly pointed out by Hochstetter (1916) and as indicated in our figure 10.

The first steps in this cleavage are well under way in embryos 14 mm. long. An embryo of this stage (Carnegie Collection, No. 940) is shown in figure 1. The veins of the head were distended with a natural blood injection and at the same time the surrounding tissues were quite transparent; as a result, it was possible to determine their arrangement from a surface examination with considerable detail. A photograph was made of the specimen, and in it were added the details that could be seen with the aid of a binocular microscope. It was in this way that figure 1 was obtained. What is seen there is the primary head-vein and its tributaries. To see the deeper structures it was found necessary to make a

plastic model of the region. Such a model is shown in figures 24 and 25, plate 3, being a wax-plate reconstruction made from an embryo 11.5 mm. long (Carnegie Collection, No. 544). Mall (1905) has pictured about the same stage in his figure 9, and this stage is also pictured by Markowski (1911) in his figure 1.

In their main points all of these embryos correspond rather closely, and apparently the vascular system at this time does not show any great variation. A large venous channel is formed in the region lateral to the diencephalon and passes

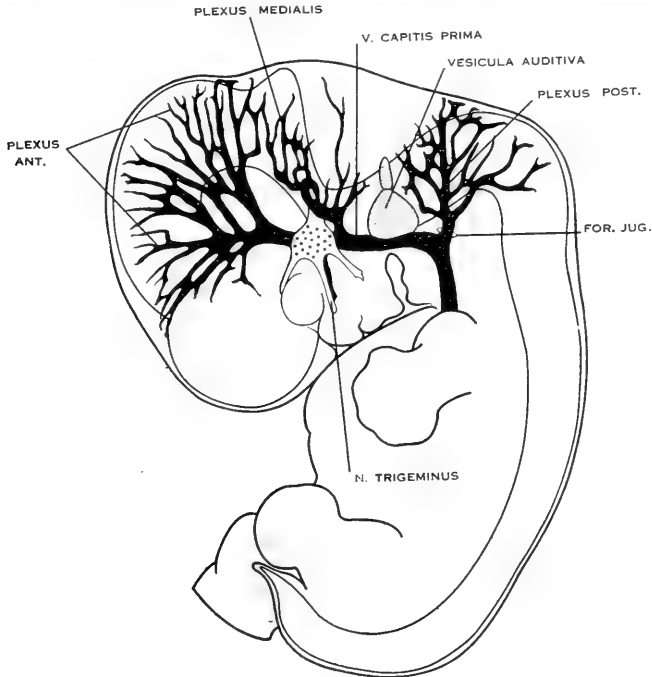


FIGURE 1.

Drawing of the primary head-vein and its main tributaries in a human embryo 13.8 mm. long (Carnegie Collection, No. 940). The tributaries are arranged in the form of three plexuses—the anterior, middle, and posterior dural plexuses. The trunk of the posterior plexus passes through the foramen jugulare in order to empty into the primary head-vein, and this point marks the junction of the intrinsic head portion of the primary head-vein with the neck portion or anterior cardinal vein. The latter eventually becomes the internal jugular vein. Enlarged about 10 diameters.

backward median to the trigeminal nerve and lateral to the otic capsule through the region of the future middle ear, where it bends sharply downward in the neck region to finally empty into the duct of Cuvier. All the veins of the cranial region drain into this main channel. This constitutes the *primary head-vein* with which we are already familiar. This vein at 14 mm. differs from its condition at 4 mm. only in being more deflected in its course by the structures through which it threads its way. It still forms a fairly direct and efficient drainage-channel. It was this

primary head-vein that was described by different writers as the anterior cardinal vein until Grosser (1907) showed that only the caudal portion of it—the part that is found in the region of the somites and later forms the internal jugular vein—could be properly spoken of as the anterior cardinal. Salzer (1895) designated the portion in the presegmental region in the guinea-pig as *vena capitis medialis* and *vena capitis lateralis*, depending on whether it was found median or lateral to the cranial nerve-trunks. The more cephalic portion, in the trigeminal region, is always found median to the nerve and hence is always *vena capitis medialis*. Caudal to the trigeminal nerve, Salzer describes it as at first coursing medial to the facial, glossopharyngeal, and vagus nerves, and subsequently, by a process of “island formation,” migrating lateral to these same nerves—that is, changing from *vena capitis medialis* to *vena capitis lateralis*. These terms were advocated on the basis of an homology with similar veins in the lower vertebrates, and were used in the recent paper by Shindo (1915). We now know, however, from the work of Sabin (1917*a*) that what Salzer called the *vena capitis medialis* is the primordial channel of the hindbrain, whose purpose is primarily the proliferation of endothelium, and hence is not to be regarded as a pure drainage-channel. The primary head-vein is the first true drainage-channel in this region. Its composite origin has already been pointed out. It has been shown that it belongs in part to the trunk (the anterior cardinal vein) and in part is intrinsic to the head. As we shall presently see, it is the trunk portion or anterior cardinal that forms the internal jugular vein, whereas the intrinsic head portion in its more anterior segment becomes the cavernous sinus, the otic or more posterior portion (the so-called *vena capitis lateralis*) disappearing entirely and being replaced by a more dorsally situated channel.

The tributaries draining into the primary head-vein join it mainly along its dorsal margin, though there are also ventral tributaries which are especially large and numerous in the neighborhood of the optic stalk and the trigeminal ganglion, as seen in figures 24 and 25. A large plexiform sheet lies median to the maxillary trunk of the trigeminal nerve, draining the structures of the maxillary arch. It is continuous with the plexus that envelops and penetrates into the substance of the trigeminal ganglion. It is a modification of this plexus that forms the infraorbital vein and the venous plexus in the region of the pterygoid fossa. The ophthalmic vein corresponds to the ventral tributaries just in front of this and median to the first division of the trigeminal nerve. This is contrary to the view of Markowski (1911, p. 600), who thought that it was the more caudal and larger of these tributaries, the one draining the maxillary process, that becomes the ophthalmic vein; whereas the more anterior tributaries, arising from the orbital fossa, he described as undergoing retrogression and disappearing. That it does not disappear, but forms the main portion of the ophthalmic vein, we shall be able to see in older stages.

The tributaries draining dorsally into the primary head-vein are arranged in three plexiform groups, as was pointed out by Mall (1905), the first group emptying into the main channel in front of the semilunar ganglion, the second group between the semilunar and the acustico-facial ganglia, and the third group caudal to the otic capsule. These were designated respectively the *anterior*, *middle*, and *pos-*

terior cerebral veins. The last one empties into the main channel through a single trunk, but the other two groups tend to maintain the character of the original plexus and usually have multiple openings into the primary head-vein. Furthermore, due to the cleavage effect of the dura, which tends to separate them from the deeper vessels, the veins forming these three groups belong chiefly to the dura mater and the tissues forming the membranous cranium. There is, therefore, an advantage in adopting for the temporary description of this period of development a terminology something like that of Markowski (1911). In doing so, a distinction between the lateral and mesial portions will not be made, but the three groups as given by Mall will be retained. We shall thus speak of the *anterior, middle, and posterior dural plexuses*, or (more formally) *plexus duræ matris anterior, plexus duræ matris medialis, and plexus duræ matris posterior*, as they are indicated in figures 1, 24, and 25. In these figures only the larger channels of the plexus are shown and it is to be understood that an intervening smaller venous mesh connects them more or less completely.

These three plexuses are not exactly uniform as regards their pattern, but from the very first they exhibit an individuality that seems to correspond to the difference in structure of the areas which they drain. The anterior plexus is modified along its oral margin in adaptation to the form of the bulging hemisphere. The middle plexus has two larger trunks, one of which drains the capillaries on the oral surface of the cerebellar plate; the other drains the anterior part of the roof of the fourth ventricle. It is this latter one that was pictured by His (1904) and incorrectly described as the sinus transversus (p. 121). In the posterior plexus there can be recognized usually a single channel that is larger than the others and that tends to cross the dorso-median line to anastomose with the plexus of the opposite side, the main channel of the other side being correspondingly smaller, thus giving rise to a bilateral asymmetry. Another place at which a larger channel is frequently seen crossing dorsally over the median line in an asymmetrical manner is at the junction of the midbrain and hindbrain. A third favorable place of this kind is over the diencephalon along the caudal margin of the cerebral hemisphere. Here is formed the beginning of the transverse sinus, which consequently shows an asymmetrical relation to the superior sagittal sinus, as will be pointed out later.

In the ventral portions the dural plexuses are more or less completely separated from the deeper-lying plexus or capillary sheet that closely invests the wall of the neural tube, in which are developed the cerebral veins and main arterial supply. In tracing the plexus dorsalward toward the median line, we find an increasing frequency of communication between the two, and near the median line they are so intimately connected that it is impossible to distinguish between them; in other words, in this region the cleavage between these two layers is not yet established. The manner in which the tips of the dural plexuses communicate with the capillary sheet of the brain-wall is indicated in figures 24 and 25, where a portion of the sheet is drawn in over the occipital pole of the cerebral hemisphere. It is to be remembered that a capillary meshwork of this kind invests the central nervous system everywhere, the pattern of the mesh varying somewhat according to the region.

The main arterial trunks are well established at this stage and afford a more abundant supply of blood to the brain than existed in the 4 mm. embryo. Whereas the aortic system in the latter conformed to the branchial arches, it now presents a definite aortic arch derived from the truncus arteriosus and the fourth branchial arch of the left side. The innominate artery is formed by the fourth arch of the right side. The third arch has been taken up on each side in the formation of the common carotid and its bifurcating portion, including the plexiform external carotid. The internal carotid, basilar and vertebral arteries are present in practically the adult form. It was noticed in studying the left vertebral artery in this specimen that its communication with the aorta was more caudal than one would expect, judging by its position in the adult. It is probable, however, that the arrangement is a temporary one, and that one of the communications above this, too slender to model, is destined to become the final trunk of the vertebral artery, thus affording an instance of spontaneous migration of a blood-channel.

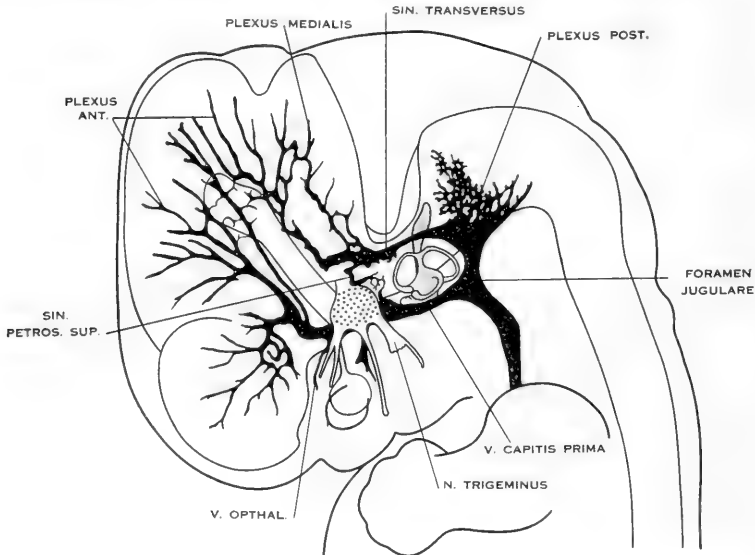


FIGURE 2.

Profile reconstruction of the dural venous system in a human embryo 18 mm. long (Carnegie Collection, No. 144). The primary head-vein is still intact; a more dorsal channel, however, is forming through the meshes of the middle dural plexus, coursing backward into the posterior plexus. This new channel becomes the permanent sinus transversus by which the greater part of the brain is finally drained through the foramen jugulare. A course completely intracranial (sinus transversus) thus replaces one that was in part extracranial (primary head-vein). Median to the second division of the trigeminal nerve can be seen the plexiform maxillary vein, which drains the structures of the maxillary process. Enlarged 13 diameters.

Similarly, as was seen in the 4 mm. embryo, the distribution of the arterial supply and the arrangement of the venous drainage are here (in the 14 mm. embryo) efficiently laid out from the standpoint of the structures of the head as then existing. The primary head-vein is already somewhat bent out of its course by the ear-vesicle and nerve-trunks, and would necessarily become much more so by the

growth that rapidly follows were it not that a new provision is established to take care of this, as we shall now see.

ADJUSTMENTS OF BLOOD-CHANNELS DUE TO GROWTH AND CHANGE IN FORM OF THE BRAIN AND EAR

Before the cleavage between the dural and cerebral vascular systems is completed, certain alterations in their pattern, associated with the changes in their environment, rapidly follow one another. If one examines a number of series of about the same age as that just described, and a little older, it is seen that the primary head-vein maintains the same general course and relations, but the pattern of the dural plexuses is constantly changing, which in the end results in a change in the primary head-vein itself. In embryos about 18 mm. long an

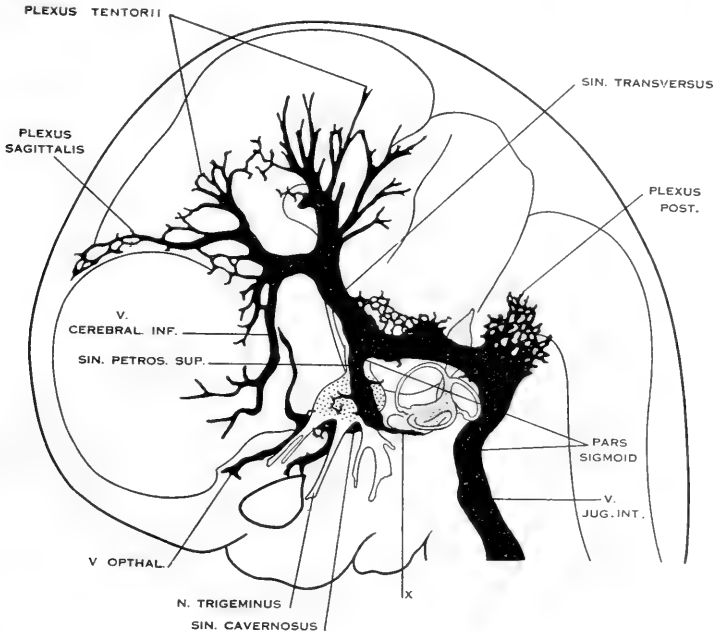


FIGURE 3.

rofile reconstruction of the dural venous system in a human embryo 21 mm. long (Carnegie Collection, No. 460). The sinus transversus is now clearly established and there is left of the primary head-vein only that portion which persists as the sinus cavernosus. There is a remnant of its otic portion (marked x) that originally connected the cavernous region with the internal jugular vein. A more complete reconstruction of the blood-vessels of the right side of this same embryo is shown on plate 4. Enlarged about 11 diameters.

important change occurs by which the blood from the middle dural plexus, which heretofore had drained into the primary head-vein, in the interval between the trigeminal and the acustico-facial ganglia, now drains caudalward into the posterior dural plexus through anastomosing loops that exist between these two plexuses, passing dorsalward to the otic capsule and just lateral to the endolymphatic sac.

This can be seen in figure 2, which shows a graphic reconstruction of a human embryo 18 mm. long (No. 144, Carnegie Collection, CR length 18 mm. in formalin, 14 mm. on slide). This is the same embryo shown in Mall's figure 11 and is about the same age as the embryo pictured in figure 2 of Markowski. In some respects the reconstruction referred to differs from both of these. From Mall it differs in that the greater part of the midbrain and forebrain is still drained by the primary head-vein. From Markowski it differs in that in our specimen the single large channel passing backward from the anterior and middle dural plexuses is not yet established, but instead the region occupied by the anterior and middle plexuses still shows an extensive anastomosing network not differing much from the pattern we have already seen in figures 1, 24, and 25. The basis for this new channel from the middle to the posterior plexus, dorsal to the otic capsule, already existed in slightly younger stages (fig. 25) in the form of a venous plexus extending across this region. We are not to conclude, however, that this plexus was placed there for this particular purpose; it is only such a plexus as tends to form everywhere throughout the dural system.

An interesting feature in connection with the establishment of the new channel just described is the fact that the trunks that originally drained the middle dural plexus into the primary head-vein nearly disappear, owing to the fact that the blood that they heretofore carried—*i. e.*, from the cerebellar region and the posterior part of the midbrain—adopts the new channel that is formed dorsal to the otic capsule and is thus drained into the posterior dural plexus. As a result of this the original trunks that connected the middle plexus with the primary head-vein become relatively small and partially break up into a small plexus. We shall see later, however, that with the next change in the head-vein a trunk will open up here again as an important channel.

In taking up the question of terminology for figure 2, it is found that most of the terms used in figures 1, 24, and 25 are still applicable. There are the three dural plexuses draining into the primary head-vein and also the ophthalmic and maxillary veins. The anterior dural plexus, however, can be seen to be reshaping itself so as to come into a more free anastomosis with the middle dural plexus. The middle dural plexus, by draining as it does over the otic capsule, presents the first stage in the formation of the transverse sinus—that is, the sigmoid portion of it. The posterior dural plexus shows less change in its form and connections than any other group of the head-veins, and this is true also in the later stages. There are some minor alterations in its pattern, but otherwise it simply extends to become the occipital sinus of the adult. The primary head-vein can be subdivided into the trigeminal portion that is to form the cavernous sinus and the otic portion which passes lateral to the otic capsule accompanying the seventh nerve, and lastly, the cervical portion or internal jugular vein, the boundary of which is indicated in figure 2 by the label *for. jug.* The otic portion already shows a diminution in volume as a result of the establishment of the new drainage-channel dorsal to the otic capsule. Dorsal to the otic capsule there is sufficient free space for the development of a vascular channel, whereas the region ventro-lateral to it becomes

crowded by the development of the cochlea and the structures of the middle ear. This constitutes a mechanical factor that doubtless has a determining influence upon the change in the course of this blood-channel.

In embryos about 20 mm. long the veins of the head have an arrangement that is intermediate between the embryonic type and the adult type. The veins in the basal portion of the skull closely resemble those of the adult, while the dorsal veins still have many embryonic features. In figures 3 and 26 are shown reconstructions of the head of such an embryo (Carnegie Collection, No. 460, 21 mm. long). Figure 3, showing left side of head, is a profile reconstruction, and figure 26, showing right side of head, is from a wax-plate reconstruction. The reconstruction of the blood-vessels in this case was greatly facilitated by the work already done on the head of this embryo by Professor Lewis, who kindly put all of his tracings and photographs at my disposal. The study was further facilitated through the fact that the blood-vessels had been injected through the umbilical vein with India ink by Professor Sabin while the heart was still beating, so that there is a beautiful injection of the entire vascular system. Before the embryo was cut, sketches and photographs of the vessels that could be seen from the surface were made by Professor Evans. For the sake of comparison another embryo slightly older (Carnegie Collection, No. 632, 24 mm.) was studied, and a profile reconstruction of it is shown in figure 4.

On examining figure 3, showing left side of the head, it is seen that the primary head-vein is now separated into its adult parts. In the trigeminal-nerve region we can speak of it as the cavernous sinus, receiving as tributaries the ophthalmic and maxillary veins and a large cerebral vein draining the lateral wall of the diencephalon. This latter vein belongs to the cerebral-vein system, eventually becoming the middle cerebral vein, and runs for the greater part of its course through the pia-arachnoid membranes. It penetrates the dura and runs a short dural course before joining the cavernous sinus. It may be regarded as one of the diminishing number of channels that drain the cerebral venous system into the dural system. There are also smaller tributaries from a network in the region of the semilunar ganglion. No tributaries were detected flowing into the cavernous sinus from the caudal pole of the cerebral hemisphere, such as were found up to this time; all of this blood now flows in the opposite direction, caudalward through the middle dural plexus and the developing transverse sinus. On the right side of this same embryo (see fig. 26) a communication still exists between the cavernous sinus and the anterior dural plexus, though it is thinning out. In this respect, then, figure 26 is just before figure 3, and a comparison of the two shows just how this interesting reversal of the blood-current takes place.

Tracing the cavernous sinus backward, it can be seen that the interruption between it and the internal jugular vein is complete, though there is still a remnant of that connection which extends as a blind channel a short way along the facial nerve. It is interesting to note that there is occasionally found in the adult skull a persistent foramen, the *foramen jugulare spurium* of Luschka, which corresponds to the exit of this decadent channel. The vein itself, however, has never been described as persisting, although it exists normally in lower forms as a drainage for

the anterior part of the brain, passing through this extracranial course to empty into the internal jugular vein. In the stage we are studying the drainage of the cavernous sinus is upward over the semilunar ganglion into what may now be recognized as the transverse sinus. This communication is through a short channel that approximately represents the original trunk of the middle dural plexus and constitutes the superior petrosal sinus. This channel is designated by Markowski (1911) as the *vena prootica*, and he gives a different origin for the superior petrosal sinus. According to him (p. 599), it takes its origin from a small cerebral vein derived from the basal surface of the hindbrain which empties into the *vena prootica*. In the further development, the opening of the vein migrates by anastomosis along the *vena prootica* toward the sinus transversus and empties either into that sinus or into the *vena prootica* near it. According to Markowski, the superior petrosal sinus has little connection with the cavernous sinus and morphologically represents a metencephalic vein. Regarding the eventual fate of the *vena prootica*, he has apparently made no observations, though he pictures it as a large channel in an embryo 46.5 mm. long. From the specimens I have examined I can not confirm Markowski's description of the superior petrosal sinus, and I feel convinced that his *vena prootica* and the superior petrosal sinus are one and the same thing, and that which he regards as the superior petrosal sinus is, instead, one of its tributaries. Mall (1905, p. 17) also described the superior petrosal sinus as the adult form of the "*vena cerebialis media*," which, it will be remembered, is the same as the trunk of our middle dural plexus.

With the alterations in the primary head-vein the anterior, middle, and posterior dural plexuses are drained by means of the new dorsal channel which empties through the jugular foramen into the internal jugular vein. This channel can be at once recognized as the transverse sinus, and the sigmoid portion of it presents relations that are much the same as are found in the adult. The three dural plexuses are still of the embryonic type. The posterior or occipital plexus is practically the same as was seen in 18 mm. embryos. Only its coarser meshes are shown in figure 3. It is more completely shown in figure 26. It will be noted that it is rather of a different character from the now combined anterior and middle dural plexuses and (as we shall later see) it is to take less part in the further metamorphosis of these vessels.

The whole dural area lying between the cerebral hemispheres and the margin of the cerebellum constitutes the tentorium cerebelli. It is very broad dorsally and is more constricted ventrally; thus in profile it is wedge-shaped. In the loose tissue composing it are found the meshes of the dural plexus, the combined anterior and middle plexuses. As this region becomes more compressed, consequent upon the growth of the cerebrum and cerebellum, there is a continual adjustment of the contained venous channels with repeated alterations in the pattern of the meshes. In general we find the larger channels radiating upward toward the midbrain region, and as we approach the median line the plexus becomes finer and there is an intimate anastomosis with the subjacent plexus belonging to the brain-wall,

On comparing embryos 21 mm. long with those 18 mm. long two characteristic changes are observed in the pattern of the anterior dural plexus at this time. (Compare figs. 3 and 26 with fig. 2). In the first place, the anterior dural plexus annexes itself to the middle dural plexus and drains backward through this into the newly established channel dorsal to the otic capsule. We will therefore, from now on, refer to the combined anterior and middle dural plexuses as the tentorial plexus, on the basis of its distribution, which is probably a more satisfactory terminology than to group both of them under the designation anterior dural plexus, as was done in the former paper (Streeter 1915). In the second place, there is differentiated along the margin of the cerebrum and between the hemispheres a subdivision of this plexus that is eventually to constitute the superior sagittal sinus (marked plexus sagittalis in figs. 3, 4, and 26).

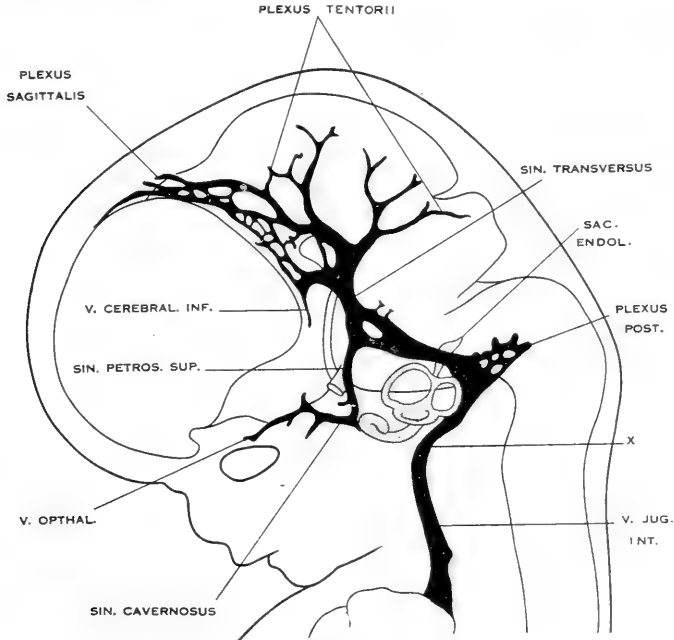


FIGURE 4.

Profile reconstruction of the main dural veins in a human embryo 24 mm. long (Carnegie Collection, No. 632). The sigmoid portion of the transverse sinus can be identified as that part between the point of entry of the superior petrosal sinus and the jugular foramen (marked X). Enlarged about 5 diameters.

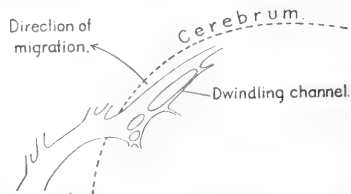
Examination of photographs and sketches of embryos of about this age shows that there is a tendency to the formation of a larger channel along the anterior margin of the tentorial plexus—that is, along the caudal margin of the cerebrum. This was designated by Markowski (1911) as the *anterior marginal vein* (vordere

(Grenzvene): the large tributary, draining the lateral surface of the cerebrum, that empties into it, he calls the *lateral telencephalic vein*, of which there may be several. Markowski describes the anterior marginal veins of the two sides as extending forward and toward the median line and uniting in the formation of a plexus out of which is to be derived eventually the superior sagittal sinus. From examination of figures 2, 3, and 4 it can be seen that there is no sharp line between the sagittal plexus as described by us and the more ventral loops of what was the anterior dural plexus of which it is a part. The *anterior marginal vein* of Markowski is a part of both of them, as can be plainly seen in figure 26. The discussion regarding the formation of the superior sagittal sinus will be reserved for a subsequent part of this paper. We may, however, point out at this time that the anterior marginal vein of Markowski is apparently not a definite vein, but rather a constantly changing channel. What we find is that the more anterior loops of the tentorial plexus are constantly dropping out and are replaced by the development of the more caudal channels. By comparing figures 3 and 4 we can see this change occurring. Our interpretation of the condition found in figure 4 is that what had been a larger channel along the cerebral margin of the tentorial plexus is now dwindling into a small mesh, whereas the main blood-stream forms for itself a new course in a more caudal loop of the plexus.

In this connection it may be pointed out that "migration of veins" may occur in at least two ways. There may be a passive change in position or direction of the endothelial tube itself, due to mechanical causes arising from alterations in its environment; this is illustrated by the sigmoid portion of the transverse sinus and its change in form in the later stages (embryos more than 20 mm. long). On

FIGURE 5.

Camera-lucida sketch of a portion of the right transverse sinus in a human embryo 27.5 mm. long (Carnegie Collection, No. 1458). It illustrates the manner of channel-migration in the loops of the tentorial plexus, whereby the main channel follows a course in adaptation to the caudal growth of the cerebral hemisphere. While this process is under way there are found along the cerebral margin the slender remnants of discarded channels.



the other hand, a vein may change its position by forming or adopting a new endothelial channel and at the same time relinquishing its original endothelial channel. The embryonic plexiform character of the veins in the region of the tentorium is especially favorable for this procedure, and we find this type of alteration in the blood-channels repeatedly illustrated in this region. In other words, under migration of veins we are to distinguish between *passive migration* (where there is a change in position due to some flexion or traction on the vein-wall itself) and *spontaneous migration* (where there is a change in position of the blood-stream only), and where, by a process of what might be called circumfluent anastomosis or anastomotic progression, the blood-stream develops a new channel in the adjacent loops of the plexus, with a corresponding dwindling of the previously used loop, as is illustrated in figure 5. From the observations of Evans (1912) on the ventral

branches of the aorta, it is apparently possible to obtain a spontaneous migration without the aid of collateral loops. Here the result is obtained by unequal growth of the endothelial walls. As a subhead under spontaneous migration we might include *replacement channels*. In this process there is the formation of a new channel and the obliteration of an old one. A replacement channel differs from other spontaneously migrating channels in that it is not a gradual and progressive change in position, but an abrupt and immediately complete one. Furthermore, the new channel lacks the morphological characteristics of the old one. An illustration of a replacement channel is the channel dorsal to the otic capsule (transverse sinus), which supplants the otic portion of the primary head-vein.

The lateral telencephalic veins of Markowski apparently correspond to the inferior cerebral veins of the adult, so we will label them in that way. Though emptying into the dural system, they develop their course through the intradural membranes and become typical cerebral veins. It is interesting to note that in the 21 mm. embryo certain definite topographical points in the transverse sinus are already determined, namely, the jugular foramen, the location of the endolymphatic sac, the points of entry of the superior petrosal sinus and of the inferior cerebral veins. Thus we see that more than half of the sinus is already established and that it is the terminal or jugular portion that is established first. The remainder of the sinus is relatively late in assuming a permanent form, which is doubtless the result of the prolonged period of growth of the cerebrum, making a continued adjustment of the tentorial plexus necessary. Even in embryos 50 mm. long, which we are about to examine, the proximal end of this sinus is still in the formative stage. Before leaving this stage, and once more comparing figures 3, 4, and 26, it should be pointed out that the great drainage-channels of the head are efficiently adapted to the drainage of its parts as then existing. We are not to think of them as busily engaged in building the transverse and sagittal sinuses, but as carrying on their functional activity in the best manner possible for the moment and with regard to the available space and the amount of given work. The completed transverse and sagittal sinuses will come in good time, as determined by later conditions.

To cover the period of embryos about 50 mm. long, the writer examined four series belonging to the Carnegie Collection: No. 886, 42 mm. coronal; No. 84, 50 mm. transverse; No. 96, 50 mm. sagittal; and No. 448, 52 mm. sagittal, injected. There was also an embryo of about the same age (No. 458, 54 mm.) that had been injected with India ink, the head of which was removed and partly dissected, and then cleared after the Spalteholz method. This gave excellent total views of the blood-vessels. The profile reconstruction shown in figure 6 is based on series No. 96 and was made by preparing tracings on transparent paper which were then superimposed and a composite tracing made of the whole series. This is about the same stage that is shown by Markowski in his figure 4. The reconstruction shown in figure 27 is of a younger embryo and was made after the Born-Lewis method.

At this period the arterial supply and the venous drainage of the head are established along channels that correspond fairly well to those found in the adult.

It is clearly subdivided into three separate systems: (1) the superficial system belonging to the integument and soft parts, (2) the dural system lying between the dura and bone, and (3) the cerebral system. All three are originally outgrowths of the same capillary plexus. The separation of the dural veins and the cerebral veins we have traced through step by step. The superficial vessels in embryos 20 mm. long are already separated off from the dural system by the membranous and cartilaginous cranium. They appear first in the lower parts of the head, where, in consequence of the earlier maturation of this region, they are originally separated off from the deep system and are in the form of a plexus that gradually spreads upward over the vault. They maintain a few anastomoses with the dural system, which constitute the so-called emissary veins. One of these is shown in figures 6 and 27. Aside from the channel maintained through the orbit, the chief drainage from the superficial system is through the external jugular vein, which is pictured by Salzer (1895) as already present in guinea-pig embryos 20 mm. long.

On examining the dura in embryos 50 mm. long it will be seen that for the greater part it closely invests the interior of the developing cranium and is relatively poor in blood-vessels. This is true especially in those portions where the cartilaginous and bony cranium is more advanced in its differentiation, as in the base of the skull and in the frontal, temporal, and lower occipital regions. In other regions the dura projects within the cranial cavity, being separated from the future bony skull by a layer of areolar tissue, in the meshes of which are found the large blood-channels and their tributaries. The largest area of this kind is situated over the midbrain, extending from the caudal margin of the cerebral hemispheres to the cerebellum. This area extends laterally down to the base of the skull, narrowing as it does so. It constitutes what is known later as the tentorium cerebelli, and in it is included the greater part of the dural venous system. A basal extension of the tentorium widens out in the region of the semilunar ganglion and in its meshes is formed the cavernous sinus. A thinner area of the same tissue extends caudalward from the cavernous sinus, median to the otic capsule, to join the jugular region. The slender plexus of veins extending through this constitutes the inferior petrosal sinus. Along all the sinuses we find this same areolar meshwork. It is not to be confused with the developing arachnoid tissue, from which it is everywhere separated by the dura. Blood-vessels supplying and draining the brain are also found in the arachnoid at this time and in some regions they are quite numerous, such as the region of the Sylvian fissure and along the more ventral parts of the midbrain and hindbrain. These cerebral vessels are everywhere separated and distinct from the dural blood-channels, with the exception of the few points where they empty into the big dural channels, as occurs in the adult. The connection between the dural system and the cerebral system is no longer by a multiple anastomosis of small vessels, but instead by isolated larger veins.

Examination of figures 6 and 27 shows that we have in fetuses at this time an arrangement of the dural venous system that in most respects follows the adult arrangement. The cavernous sinus still has a simpler character than is found in the adult. It is situated median and ventral to the semilunar ganglion and has

the large ophthalmic and maxillary tributaries in front. In figure 27 it receives a large terminal trunk lateral to the infundibulum made up of tributaries coming from the region of the Sylvian fissure; this corresponds to the middle cerebral vein of the adult. Caudally the cavernous sinus communicates with the main blood-stream by means of the superior and inferior petrosal sinuses. The superior petrosal sinus passes over the cochlear part of the otic capsule and empties above

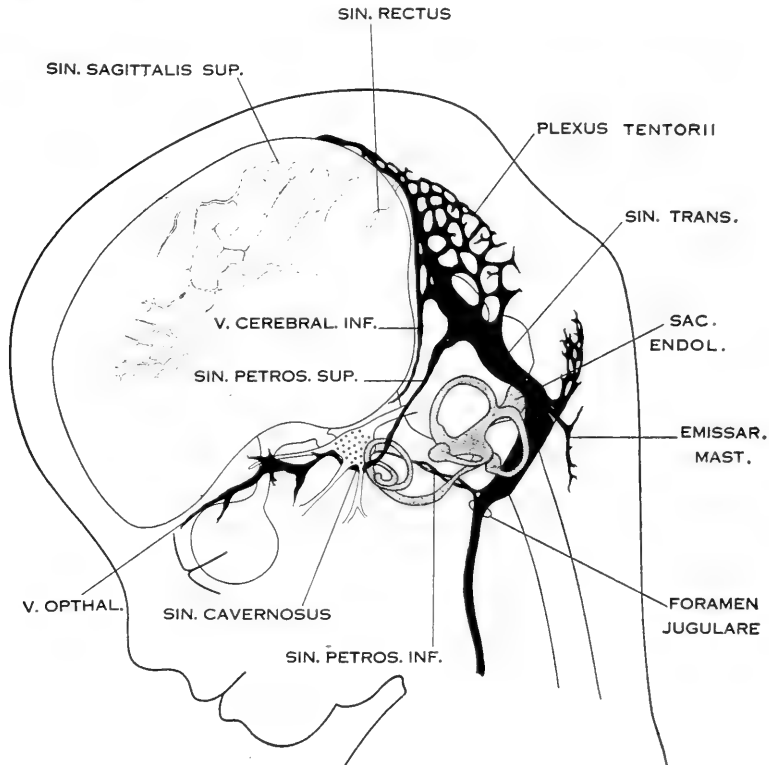


FIGURE 6.

Profile reconstruction of the dural veins in a human embryo 50 mm. long (Carnegie Collection, No. 96). The outlines of the veins of the falx cerebri can be seen through the cerebral hemisphere. That part of the transverse sinus situated caudal to the superior petrosal sinus (sigmoid) shows practically the adult relations. The more anterior part is still in the form of a plexus embedded in the embryonic tissue of the tentorium. Enlarged 4 diameters.

into the transverse sinus. The inferior petrosal sinus consists of a plexus of veins that passes median to the otic capsule to empty at the point of origin of the internal jugular vein. It is shown in figure 6 but not in figure 27.

As regards the transverse sinus, it has been pointed out that the terminal or jugular portion of it is established first. In both figures 5 and 27 it can be seen

that it consists of a single large channel from the point of entry of the superior petrosal sinus to the jugular fossa (in other words, the sigmoid portion) and has the same tributaries and the same general relations that are found in the adult. The remainder or proximal portion of the transverse sinus is less well established, and the large capillary meshwork found along its dorsal margin shows that the blood-channels here are still in the formative stage and must still be spoken of as the temporary tentorial plexus. The main channel is forming along the anterior margin of this plexus, into which the inferior cerebral vein empties. It can be seen how this portion of the transverse sinus undergoes spontaneous migration backward in adjustment to the growth of the hemisphere and thus comes to assume a more and more horizontal course. This change in direction, together with an increase in length and diameter of the main channel at the expense of the formative meshwork, remains to be completed before the adult condition can be considered as established. The variations found in the adult in the region of the confluens sinuum can be readily understood as variations in channel selection through this tentorial meshwork.

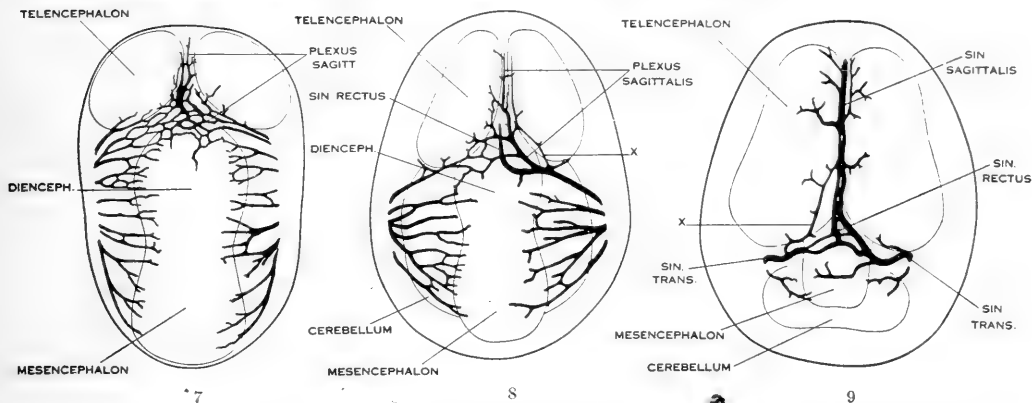
In the region of the forebrain a fold of dura is interposed between the two hemispheres and is compressed into a flattened sheet which is to constitute the falx cerebri. This and the vascular meshwork belonging to it are directly continuous with the tentorium. Like the tentorium, it passes through a prolonged adjustmental period. In embryos 50 mm. long two of its permanent channels, which are to belong to the dural sinus system, can be readily recognized; these are the superior sagittal sinus and the straight sinus. In figure 6 the superior sagittal sinus is quite irregular in outline, which is a result of shrinkage of the specimen. In the normal state, as seen in other embryos, it passes evenly along the margin of the cerebrum. Certain details regarding the vessels belonging to the falx cerebri and the drainage of the chorioidal masses will now be taken up in connection with the formation of the superior sagittal sinus.

DEVELOPMENT OF SINUS SAGITTALIS SUPERIOR.

Under the description of embryos 21 mm. long mention was made of the formation of a *plexus sagittalis* as a subdivision of the anterior dural plexus. At that stage the plexus is clearly differentiated from the remainder of the anterior dural plexus, as can be seen in the dorsal view of an embryo of about that age shown in figure 8 and in the reconstruction shown in figure 26. Earlier than this, in embryos about 14 mm. long (fig. 7), the plexus can be recognized, though here it is not so clearly separated from the general plexus. In such embryos it can be seen that the larger tributaries of the anterior and middle dural plexuses stop short of the median line, with the exception of anteriorly, where they merge into a longitudinal plexus that dips in between the developing hemispheres. It is in the meshes of this plexus that we find the beginning of the superior sagittal sinus; and the principal steps in its transformation can be seen by comparing figures 7, 8, and 9. Sketches like these necessarily have to be simplified, and on examining them it should be remembered that only the larger channels are shown and that in between

there is everywhere a fine anastomosing network. Also, the channels do not lie all in the same plane. Furthermore, it is to be noted that there exists in embryos of the same age a considerable variation in the pattern formed by these channels. The three specimens selected, however, may be regarded as illustrating fairly definite stages in this transformation.

In figure 7 is shown a dorsal view of the head of the same embryo previously shown in figure 1 (Carnegie Collection, No. 940, 13.8 mm. long). It is about the same age as the embryos shown in figures 24 and 25. Here we find the sagittal plexus represented in its simplest form. It will be noted that it possesses two characteristic features: In the first place, there is a tendency to an enlargement of



FIGS. 7, 8, and 9.—Three stages in the formation of the sagittal plexus, showing its asymmetrical character and its conversion into the superior sagittal sinus. Draining into it from below is the drainage-channel from the chorioidal bodies that is later known as the straight sinus. The channels marked x are interpreted as undergoing retrogression, being replaced by more caudal channels. Large dural channels do not persist in the areas covering the cerebral hemispheres; they are found only in the loose embryonic tissue filling the spaces and fissures that lie between the different subdivisions of the brain. Figure 7 is a vertex view of a human embryo 13.8 mm. long (Carnegie Collection, No. 940). Figure 8 is a vertex view of an embryo 20 mm. long (Carnegie Collection, No. 349). Figure 9 is a drawing of an injected and cleared specimen 54 mm. long (Carnegie Collection, No. 438) reversed.

certain portions of the plexus, irrespective of a continuous channel. We thus have a series of small lakelets connected by narrow channels. A definite single superior sagittal sinus can not yet be said to exist. In the second place, the plexus is distinctly asymmetrical and shows a tendency to drain more freely to one side than the other, in this case to the right.

A more definite and simpler channel system is found in 20 mm. embryos, an example of which is shown in figure 8 (Carnegie Collection, No. 349). Here one might possibly speak of a superior sagittal sinus. The channels, however, are still in the form of a plexus, and hence the term *plexus sagittalis* is retained. This view regarding the early identity of the superior sagittal sinus differs from that given by Evans, who pictures the primitive capillary plexus creeping up on each side of the forebrain in 8 mm. pig embryos. A portion of the dorsal margin of this plexus he labels as the primitive superior sagittal sinus (Evans, 1909, fig. 15*b*;

Evans, 1912, figs. 399 and 400). According to him it is thus originally paired and bilaterally symmetrical. According to the present writer, it is not until later that we can speak of a superior sagittal sinus. It is not until the plexuses, described by Evans, have anastomosed across the median line and have formed a longitudinal network in the meshes of which an asymmetrical channel is finally established, that we can speak of a superior sagittal sinus. In some cases two or more larger longitudinal channels are formed in the mesh, as is shown in figure 10; but in such cases they are not strictly bilaterally symmetrical.

Owing to the growth of the cerebral hemispheres in 20 mm. embryos, there is formed a well-marked cerebral longitudinal fissure which is occupied by embryonic tissue. This rapidly takes the form of the adult falx cerebri. It is in the dorsal part of this loose dural tissue that the meshes of the sagittal plexus are found.

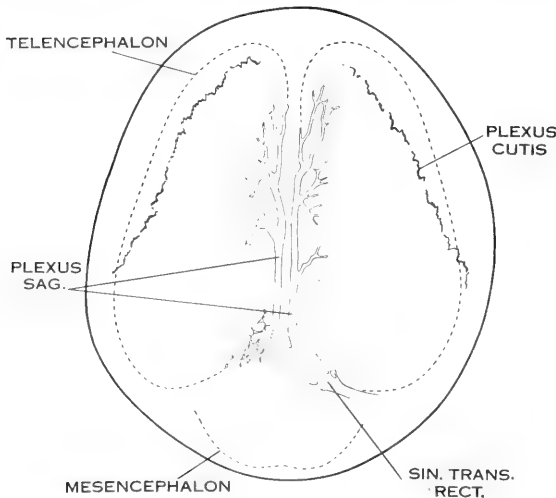


FIGURE 10.

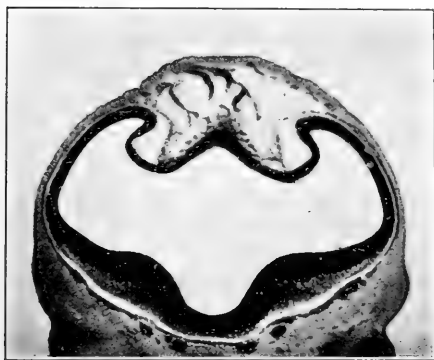
Camera-lucida sketch of the vertex of the head of a human embryo 27.5 mm. long (Carnegie Collection, No. 1458), in which the blood-vessels were conspicuous through a natural injection. It is the exception where two channels are found so nearly the same size. From a single specimen, like this, one might incorrectly gain the impression that the superior sagittal sinus is at first bilaterally symmetrical. Even here, however, it can be seen that both of the larger channels drain caudally to the right side. The minute anastomosing loops that connect the two main channels with each other and with the channels in the more ventral parts of the falx are not shown. On each side can be seen the serrated upper margin of the advancing capillary plexus of the skin. Above this margin the skin is as yet nonvascular. On account of the dilatation of these vessels along this advancing edge the margin is conspicuous.

At this time it can be seen that one or more larger channels are opening along the dorsal mid-line, which will form the superior sagittal sinus, and connected with them by anastomosing loops is a more ventrally situated large channel that constitutes the *sinus rectus*. This latter extends forward and drains the lower part of the falx. It has two converging limbs in front that drain the choroidal masses of the hemispheres. In figure 26 a portion of the right hemisphere is removed to expose the region of the falx cerebri. Here the straight sinus can be seen as differentiated out from the sagittal plexus, with which it anastomoses freely at its caudal end in a plexiform manner. Anteriorly the straight sinus bifurcates, enters the choroidal fissure on each side, and terminates in the sinus-like choroidal bodies. These, on the other hand, are fed from the caudal end by the choroidal arteries.

On coming to embryos 50 mm. long, figure 9 (Carnegie Collection, No. 458, 54 mm.), we find that here the superior sagittal sinus is established, at least in part. In its cephalic portion there is a large characteristic channel, lacking only the dural

connective-tissue investment to make it an adult type. In its more caudal portion it still exhibits a plexiform character that indicates its transitional state. Upon comparison of a number of series, the writer is led to interpret the formation of a single channel as the outcome of more than one process; in some segments there seems to be the selection of a favorable loop of the plexus which enlarges and becomes the main channel, and in other segments there is apparently an enlargement of two or more collateral loops which subsequently fuse into a more or less common channel. Both processes are apparently represented in figure 9. It is to be expected that we will find a considerable variation in this respect in different brains. In figure 10 is shown a specimen which is about the same age as that shown in figure 8. In this case two collateral channels of about equal size have formed, both draining, however, to the same side.

11



12

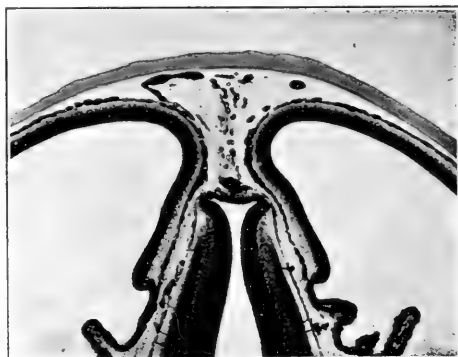


FIG. 11.—Section showing the sagittal plexus in a human embryo 14 mm. long (Carnegie Collection, No. 940, slide 15, row 3, section 1). The section shows the falciform area, the hemispheres being retracted from its lateral margins. It will be noted that there are two main plexiform vascular sheets—a superficial one near the skin and a deeper one directly against the brain-wall, the latter draining into the former by anastomosing loops. The superior sagittal sinus develops in the meshes of the superficial plexus, and the straight sinus develops in the meshes of the deep plexus over the area corresponding to the third ventricle.

FIG. 12.—Section showing the sagittal plexus in a human embryo 21 mm. long (Carnegie Collection, No. 460, slide 11, row 3, section 4), injected with India ink. The arrangements are similar, but more advanced than those shown in figure 11. The straight sinus can be recognized. The superior sagittal sinus still has the form of a bilaterally asymmetrical plexus from which a finer meshwork extends into the loose falciform tissue intervening between the two hemispheres and anastomoses with the straight sinus. On the left side a characteristic communication can be seen connecting the deep cerebral plexus with the coarser sagittal plexus above.

No attempt was made to study the histological changes that occur in the completion of the superior sagittal sinus, or of the cavernous sinus. These involve details with which the present paper is not concerned. The caudalward growth, however, of the superior sagittal sinus in adjustment to the corresponding growth of the hemispheres is of interest in our general problem. By comparing figures 7, 8, and 9 it can at once be seen that this caudal development is accomplished at the expense of the meshes of the tentorial plexus, in which process the transverse and straight sinuses also take part. These channels gradually obtain a more caudal course by what we have already described as *spontaneous migration*. The channel repeatedly shifts into a more caudal loop of the plexus, the new loop enlarging and the old loop dwindling. The veins marked x in figures 8 and 9 may thus be interpreted as discarded channels. The eventual *confluens sinuum* (torcular Herophili)

represents the point at which this caudal development reaches its completion—or, in other words, is a remnant of the embryonic tentorial plexus and usually retains a trace of the plexiform character that is found throughout the embryonic stages.

It is interesting to note that the asymmetry of the superior sagittal sinus expresses itself in the embryo as well as in the adult by a tendency to drain more to one side of the head than to the other. This becomes established by the time the embryo is 20 mm. long. The drainage is preponderantly toward the right side. It happens that in figure 9 the main drainage was in reality toward the left side. In reproducing the sketch the figure was reversed right for left, in order to facilitate its comparison with figures 7 and 8. In the accompanying table is given a list of embryos which were examined as to this point, and it will be seen that of 18 specimens all but 2 drained predominantly toward the right side, that is, about 89 per cent. In order that account should be taken of the artificial element introduced in those specimens where the vascular system had been injected with coloring matter, such specimens are indicated in the table by an asterisk. No explanation has thus far been reached to explain this interesting asymmetry. The drainage of the straight sinus could not be determined as well in the younger stages, and there were not enough of the older stages upon which to base an average. A similar asymmetry might be expected here.

SUPERIOR SAGITTAL SINUS.

No.	CR length.	Drainage outlet.	No.	CR length.	Drainage outlet.
	<i>mm.</i>			<i>mm.</i>	
296	17	Right transverse sinus.	199	35	Right transverse sinus.
350	19	Do.	*449	36	Do.
128	20	Do.	224	40	Do.
*349	20	Do.	886	43	Do.
*460	21	Do.	95	46	Left transverse sinus.
966	21	Do.	84	50	Right transverse sinus.
*382	23	Do.	96	50	Do.
*632	24	Do.	*458	54	Left transverse sinus.
145	33	Do.	613	80	Right transverse sinus.

* Injected specimens.

SUMMARY.

In describing the development of the blood-vessels of the brain the process has been subdivided into the following five arbitrary periods: (1) angioblastic period, in which the primordial vascular system becomes established; (2) resolution of the primordial system into arteries, veins, and capillaries, and the establishment of the primary type of circulation; (3) cleavage of the vascular system of the head into the external, dural, and cerebral layers; (4) adjustments of vascular channels, due chiefly to growth and change in form of the otic capsule and the brain; and (5) completion of histological differentiation of the walls of the vessels. Aside from adding perhaps more emphasis to the morphological aspects of the precirculatory type of the vascular system, the contributions of this paper are concerned only with the third and fourth developmental periods, and even there they are more or less restricted to the main drainage-channels and their gradual metamorphosis into the adult dural veins and sinuses. It has been possible to present a rather complete series of stages from which the essential factors in this process can be clearly

deduced. In order to facilitate a review of the successive steps in this interesting metamorphosis, there have been assembled on plate 1, figures 13 to 21, a series of simplified sketches, and through the aid of these it is hoped that the steps that are outlined in the following summary can be readily identified.

In the primary type of circulation the arrangement for the drainage of the capillaries of the head (figs. 13, 14) consists bilaterally of one main channel, the "primary head-vein," that starts in the region of the midbrain, runs caudalward alongside of the brain-tube, and terminates at the duct of Cuvier. The primary head-vein is composite in origin. That portion of it rostral to the vagus nerve is an intrinsic vein of the head; the remaining caudal portion is in reality a neck-vein and constitutes the anterior cardinal vein—eventually the internal jugular vein. Together these portions form a continuous channel, the primary head-vein, into which the blood from the capillary sheet immediately investing the brain-tube is drained by means of anastomosing venous loops. These loops are arranged more or less in the form of three plexuses—the anterior dural plexus, the middle dural plexus, and the posterior dural plexus. Other small tributaries which are not all shown in the figures empty into the primary head-vein, thereby draining the structures ventral and lateral to the brain-tube, such as the nerve-ganglion masses and the maxillary and mandibular gill-bars. A large one comes from the eye region and eventually is modified into the ophthalmic vein.

From this simple group of drainage channels are eventually derived all the adult venous sinuses. The metamorphosis which they undergo is based on a series of circulatory adjustments that are made necessary by certain changes in their environment, the two most conspicuous being the changes in the region of the cartilaginous capsule of the labyrinth and the still greater changes involved in the growth and marked alteration in the form of the brain. Among the factors involved in these circulatory adjustments may be mentioned the reduction of plexuses into simple channels, the conversion of channels into plexuses, the total obliteration of established channels, and the change in position of channels. Under this latter phenomenon there is to be recognized a "passive migration," where there is a change in the position of the vein-wall itself, due to the movement of its environment, which exerts a flexion or traction force upon it. We also recognize a "spontaneous migration," where there is a change in position of the blood-stream only, where in a circumfluent manner the blood-stream develops a new channel in the adjacent loops of the plexus, with a corresponding dwindling of the previously used channel. The "replacement channel" might be mentioned as another type of spontaneous migration, in which the venous channels are changed in position and direction in this process of adjustment. In the replacement channel there is the formation of a new channel and the obliteration of an old one, as in other types of spontaneous migration. It, however, differs from them in that it is not a gradual and progressive change in position, but an abrupt and immediately complete one. Furthermore, the new channel lacks the morphological characteristics of the old one. With these various factors in mind one can readily follow the steps by which the primary head-vein and its tributaries gradually merge into adult dural sinuses.

While the three head-plexuses are spreading upward (figs. 14 and 15), the outlines of the dura mater and the arachnoid spaces make their appearance, and first of all in the ventral parts. This results in a general separation or cleavage of the more superficial primary head-vein and its three tributary plexuses from the subjacent vessels that arise from and drain the capillary sheet directly investing the brain-tube. This deeper system, however, continues to drain into the former at certain places, notably in the more dorsal parts. The primary head-vein and its three tributary plexuses thus become established as a true dural system as distinguished from the deeper "cerebral veins" belonging to the arachnoid-pial membrane. The diploic veins are a later subdivision of the dural system. The superficial vessels of the head belonging to the integument and soft parts are separated off in the more ventral regions and from there spread upward over the head independently of the dural system. We then have for the head three separate systems: (1) the superficial layer belonging to the integument and soft parts; (2) the middle layer belonging to the dura and diploe; (3) the deep layer of cerebral vessels belonging to the brain. It is the middle layer, or dural system, that is exclusively concerned in the formation of the dural sinuses and whose changes in form and position we are now following.

In the region of the cartilaginous capsule of the labyrinth adaptive changes in the dural channels occur early (figs. 14, 15, and 16). Owing to the marked elaboration of these structures in this region, the course of the primary head-vein, ventro-lateral to the otic capsule, becomes an unfavorable one. If it persisted it would be tortuous and remote from the area drained; instead, this part of it becomes obliterated, and during this obliterating process an adjustment is made in two ways (figs. 14, 15, and 16): first, a channel is established in the venous plexus above the otic capsule, and through this the middle dural plexus thereafter drains caudally into the loops of the posterior dural plexus; second, the anterior dural plexus, which originally drained into the primary head-vein, completely reverses its direction of flow and drains through anastomosing loops into the middle dural plexus and through the newly established channel dorsal to the otic capsule.

In this way a complete trunk for the drainage of the head becomes established which is everywhere dorsal to the primary head-vein as far as the jugular foramen, where it is continuous with the internal jugular vein. Of the primary head-vein there is left, in addition to the cardinal portion of it or internal jugular vein, only that part in the region of the trigeminal nerve. This may now be spoken of as the "cavernous sinus." Into it drain a vein from the base of the brain and the veins from the orbital and maxillary regions; whereas it, in turn, drains upward through the original trunk of the middle plexus, which is now the superior petrosal sinus, into the newly established dorsal channel. By comparing with later stages (figs. 17 to 21) it will be seen at once that this dorsal channel is the transverse sinus, of which that part between the superior petrosal sinus and the jugular foramen forms its sigmoid portion. Thus in the 21 mm. embryo the dural channels in the region of the temporal bone have acquired essentially all their permanent connections, with the exception of the inferior petrosal sinus, which appears a little later (fig. 19).

Otherwise there remains to complete the adult condition only a certain amount of passive migration in accommodation to the changes in the adjacent parts.

The adjustment in the dural channels rendered necessary by the protracted growth of the hemispheres extend much later in fetal life. A large part of this adjustment is accomplished by spontaneous migration of the principal channels, and for this reason a venous plexus is essential. We thus find in the neighborhood of the advancing occipital pole of the hemispheres a continuous persistence of the transitory or embryonic dural plexus from which are evolved all the veins of the *falx cerebri* and of the *tentorium cerebelli*.

An anterior subdivision of the plexus extends forward in the median line as the *plexus sagittalis*, being interposed as a vertical curtain between the hemispheres. Among its dorsal meshes is developed an asymmetrical longitudinal channel which we know as the "superior sagittal sinus." In its early stages this channel is made up of several collateral anastomosing veins. The eventual single channel is formed in the anterior portions by the selection and enlargement of the most favorable vein with a corresponding disappearance of the others. In the posterior portions there is apparently some coalescence of adjacent veins. The anterior part of the sinus is completed first. As the hemispheres extend backward the sinus correspondingly elongates itself by incorporating the more caudal loops of the plexus. Transverse sections through this part of the sinus in older fetuses thus usually reveal incomplete coalescence of the separate loops. The sagittal plexus very early exhibits a tendency to drain more to one side of the head than to the other and usually toward the right side. As the superior sagittal sinus becomes established we thus find that caudalward it is usually continuous with the ventral main channel of the right anterior plexus (or tentorial plexus as it is better called in the late stages), which eventually forms part of the right transverse sinus. The straight sinus is formed in the ventral part of the sagittal plexus and its caudal adjustment is essentially like that of the superior sagittal sinus. It may drain chiefly toward the right or left plexus or equally toward both.

In embryos between 35 and 50 mm. long (figs. 18 and 19) we can recognize a main channel of the tentorial plexus that is to become the transverse sinus. If we disregard the sigmoid portion of it, it forms a fairly straight line with the internal jugular vein. In the interval between the 50 mm. embryo and the adult the transverse sinus bends backward until it comes to lie at an angle of 90° with the internal jugular. This marked change in position is accomplished in large part by spontaneous migration, by the repeated shifting back of the main blood-current into more caudal loops of the plexus, with subsequent dwindling of the discarded anterior loops. As the sinus becomes more definitely established the tentorial plexus becomes relatively smaller (fig. 20) and the final change in position is completed by passive migration, that is, actual traction on the vein-wall by its environment. In this change in position of the transverse sinus the superior sagittal sinus and the straight sinus participate and we find in the adult, at the point where they meet, an anastomosis, the *confluens sinuum* which is usually plexiform in character and represents the last trace of the embryonic tentorial plexus.

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EXPLANATION OF PLATES.

PLATE 1.

Simplified profile drawings of the dural veins, showing the manner in which they adapt themselves to the growth and change in form of the brain in human embryos from 4 mm. to birth.

Fig. 13, embryo No. 588, 4 mm.; fig. 14, embryo No. 940, 14 mm.; fig. 15, embryo No. 144, 18 mm.; fig. 16, embryo No. 460, 21 mm.; fig. 17, embryo No. 632, 24 mm.; fig. 18, embryo No. 199, 35 mm.; fig. 19, embryo No. 96, 50 mm. CR length; fig. 20, embryo No. 234a, 80 mm. CR length; fig. 21, adult.

PLATE 2.

Right and left profile views of a wax-plate reconstruction of the main arteries and veins in a human embryo 4 mm. long (Carnegie Collection, No. 588). Enlarged about 40 diameters.

This stage illustrates the character of the first type of the circulation of the head and its relation to the other blood-vessels of the body. The primary head-vein and its tributaries which form the main drainage-channels of the head are shown in blue. These communicate by anastomosing loops with the capillary plexus everywhere investing the brain-wall, only patches of which are shown in the model. The capillary plexus of the brain-wall is fed by arterial feeders, the stumps of which, as shown in the model, arise from the aortic system. The trunk that persists as the internal carotid artery is already quite definite.

PLATE 3.

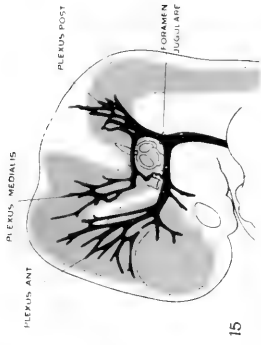
Right and left profile views of a wax-plate reconstruction of the blood-vessels of the brain in a human embryo 11.5 mm. long (Carnegie Collection, No. 544). Enlarged about 14 diameters. The primary head-vein still constitutes the main drainage-channel of the head. The manner in which its tributaries tap the deep capillary sheet investing the brain is indicated over a small area of the cerebral hemisphere. A capillary mesh of that kind invests the entire central nervous system, but is not shown in the model.

PLATE 4.

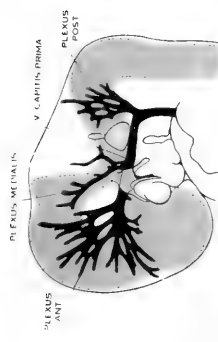
Left lateral view of a wax-plate reconstruction of the larger blood-vessels of the brain in a human embryo 21 mm. long (Carnegie Collection, No. 460). Enlarged 16.4 diameters. Instead of the head being drained by the primary head-vein, this is now accomplished by a more dorsally situated channel that has formed through the meshes of the middle and posterior dural plexuses to become the transverse sinus. (Compare with text-figure 3, which shows a left profile of the same specimen.) All that is left of the primary head-vein is that portion which is to become the cavernous sinus. In this model the right cerebral hemisphere has been dissected so as to expose the chorioidal body with its arterial feeder and the straight sinus draining it. The plexiform character of the superior sagittal sinus and of the caudal end of the straight sinus is indicative of their transitory condition.

PLATE 5.

Left profile view of a wax-plate reconstruction of the blood-vessels of the brain in a human embryo 43 mm. long (Carnegie Collection, No. 886). Enlarged 8 diameters. The vascular architecture is at this stage beginning to approximate the adult condition, although the whole tentorial region retains its embryonic character, due to which the marked subsequent migration of the transverse sinus is possible. It will be noted that the sigmoid portion of the sinus is fairly well established.



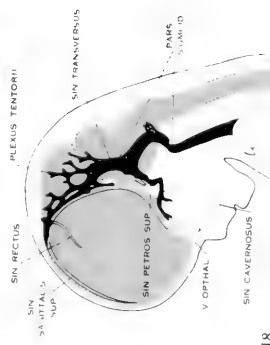
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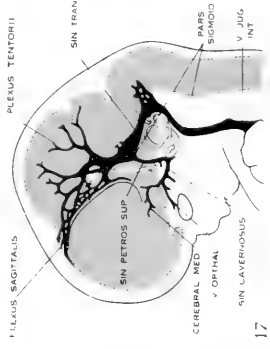
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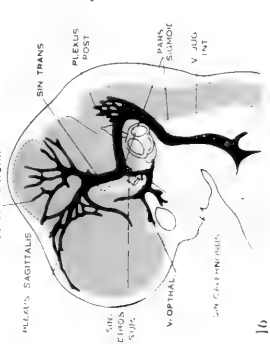
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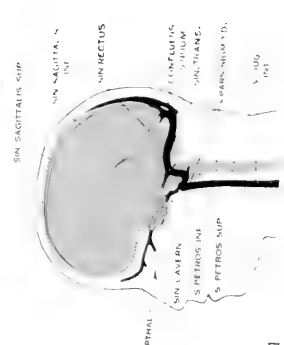
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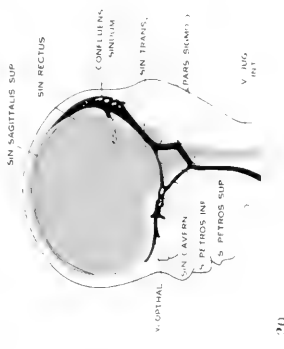
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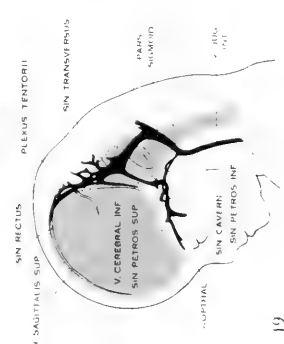
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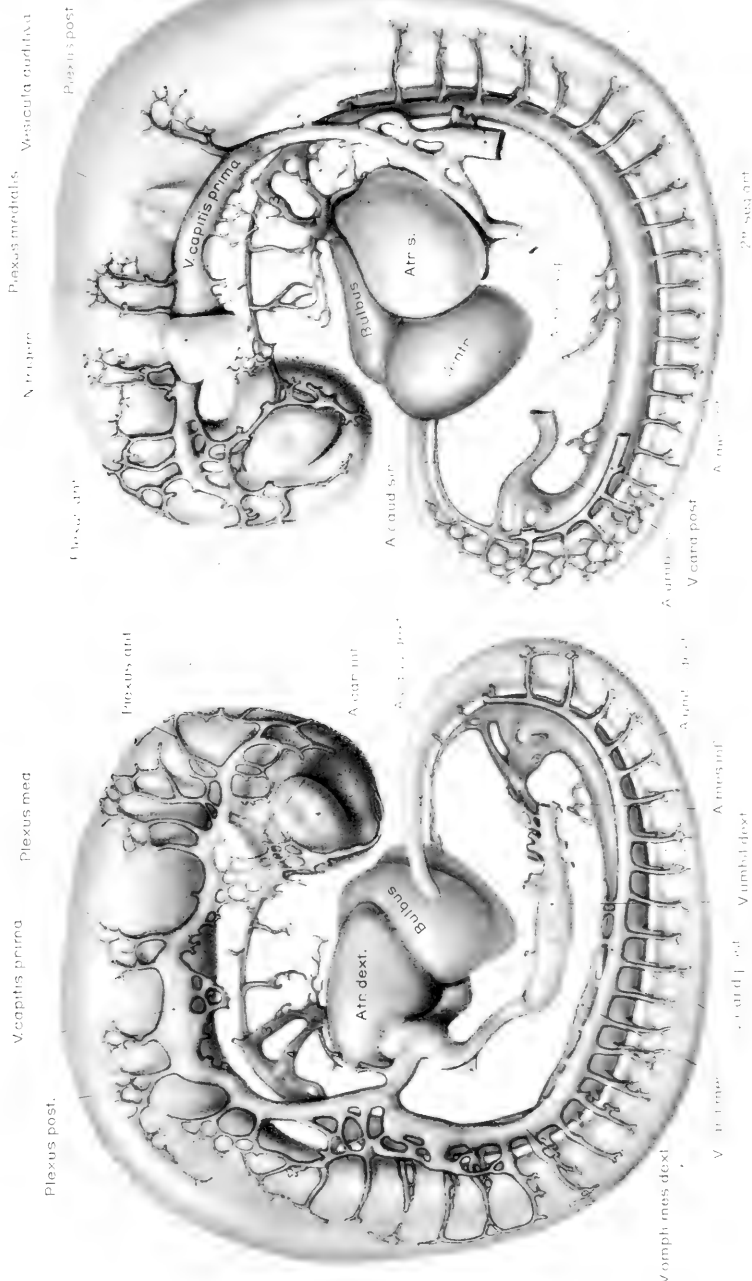


Fig. 22

Fig. 23

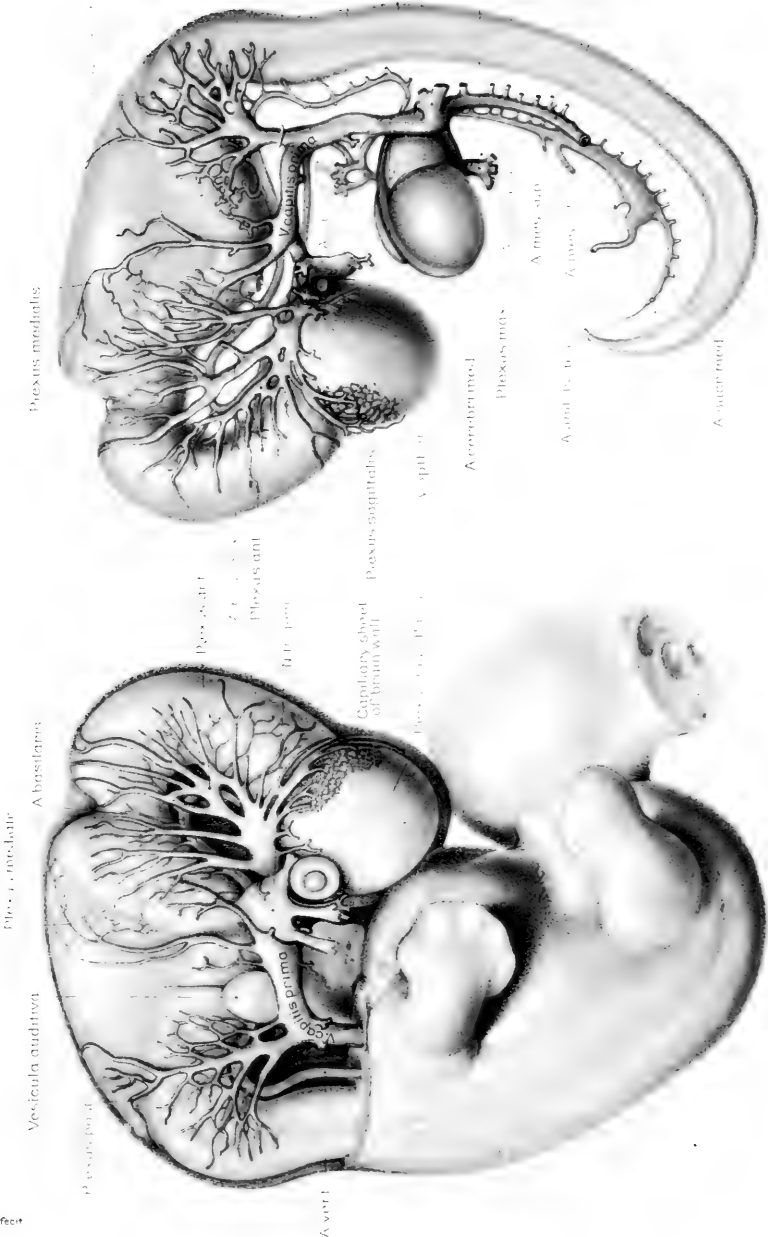


Fig. 25

Fig. 24

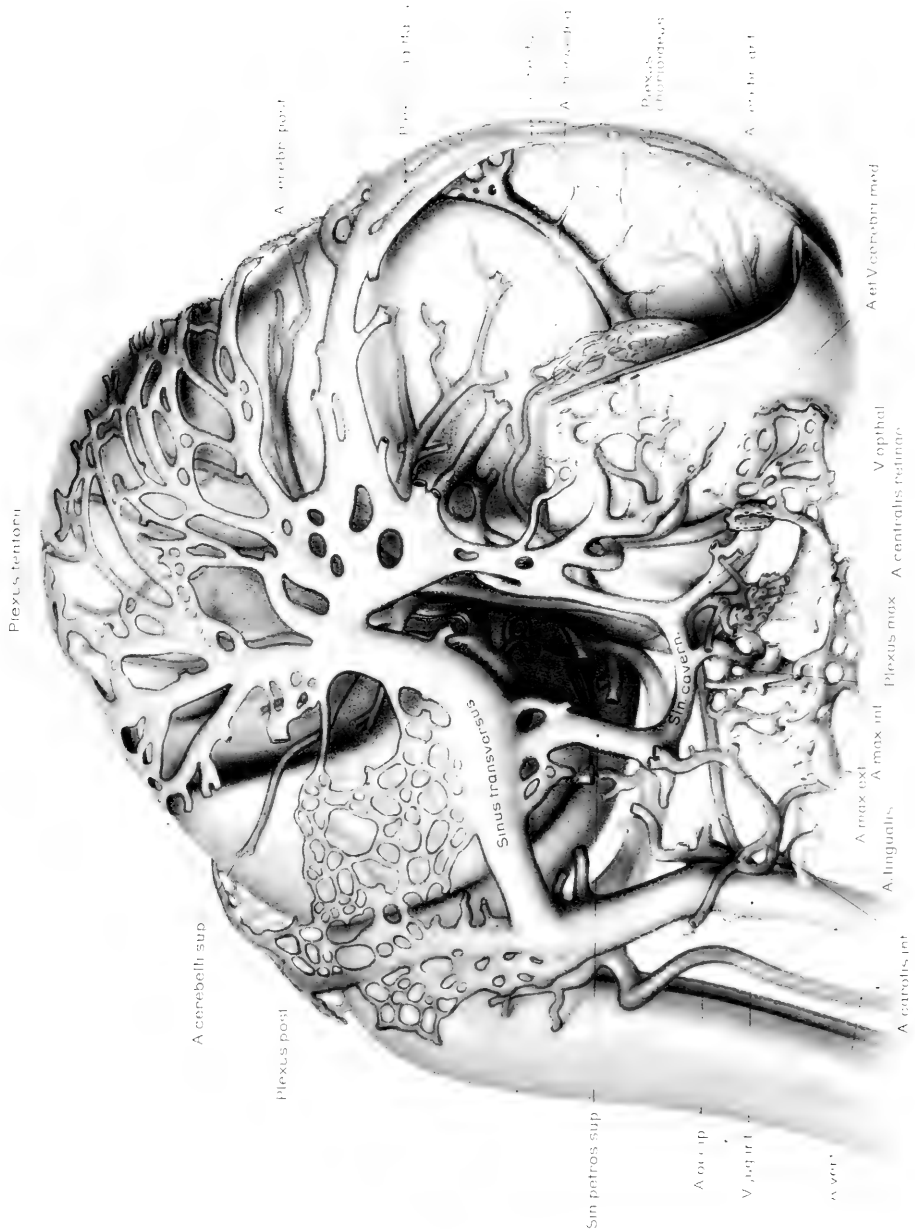
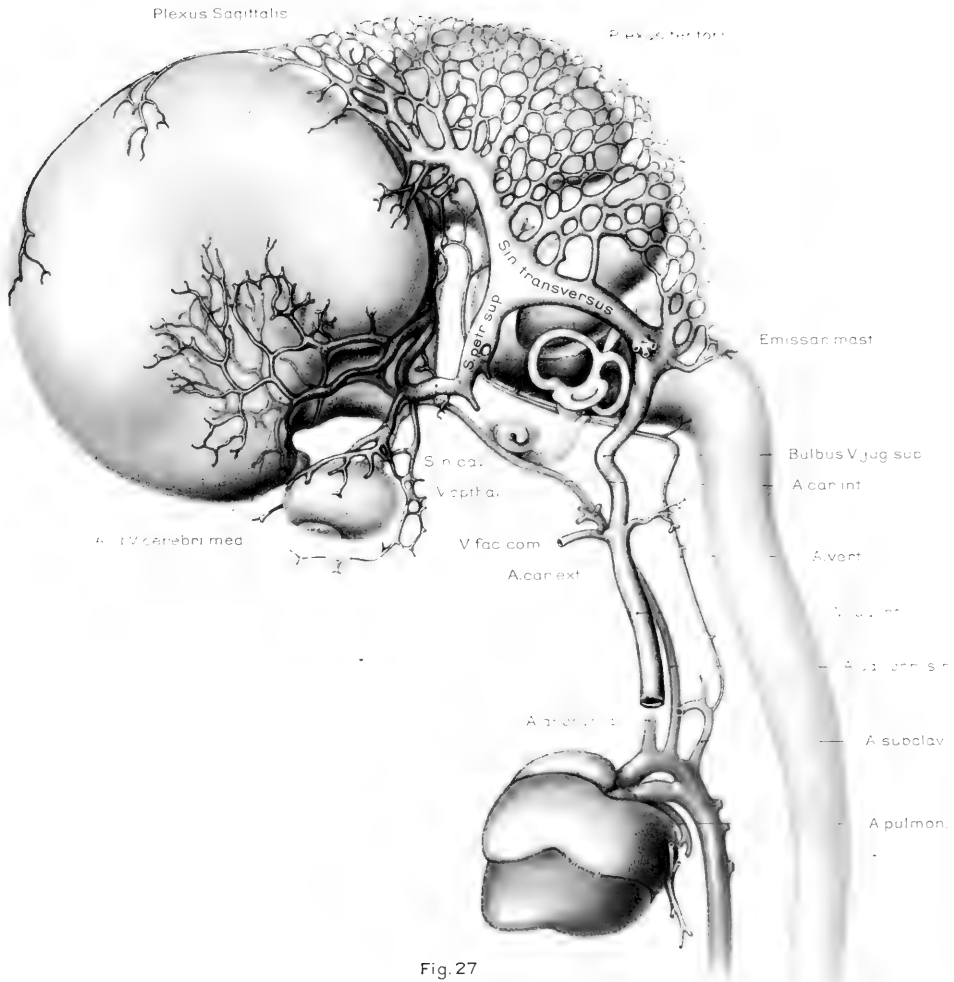


Fig. 26



CONTRIBUTIONS TO EMBRYOLOGY, No. 25.

THE MITOCHONDRIAL CONSTITUENTS OF PROTOPLASM

By E. V. COWDRY.

One plate and nine text-figures.

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THE MITOCHONDRIAL CONSTITUENTS OF PROTOPLASM.¹

BY E. V. COWDRY.

INTRODUCTION.

Schultze defined the living substance, protoplasm, as being a glass-like, semi-viscid material in which granules are embedded. We have been permitted to go a step further, because we can now recognize among these granules a definite class, which we call mitochondria and which, within a surprisingly short space of time, have been described in the cells of all tissues, in the adult condition and in all stages of development. They have been found in rapid succession in all organisms which have been studied, from the protozoa to man, and from certain of the algæ to the highest of plants. It has become apparent that they are inseparable from living protoplasm and that they yield place to no other visible constituent of living matter in breadth of distribution. Their characteristic form (strongly suggestive of bacteria), their lipoid properties, and their extraordinary sensitivity to certain types of pathological change have become familiar. These and other discoveries have stimulated interest in many quarters and have given rise to hastily conceived and poorly supported theories and to much discussion, with the result that ideas of cell structure have been, in large measure, recast.

Recognizing the importance of this new line of work, I have attempted to set forth our present knowledge of mitochondria and their significance from the standpoint of cell development and cell function, making use of unpublished observations of my own and of the literature on the subject. In a field so large it has been necessary to choose and select, to elaborate some points and to leave others almost untouched, so that many important contributions have been passed without mention. At first the researches on mitochondria were purely descriptive, but now they have taken an experimental turn, and it is this aspect of the subject which I venture to emphasize.

From the beginning, the Department of Embryology of the Carnegie Institution of Washington has placed valuable apparatus at my disposal. The work was carried on in the Anatomical Laboratory of the Johns Hopkins University, and, during the summer of 1916, at the Marine Biological Laboratory, Woods Hole. It has been completed through the action of the Peking Union Medical College in giving me the freedom and the time necessary during the past year.

I. HISTORICAL REVIEW.

It is impossible to say who first discovered mitochondria, for, with the outburst of interest in cell granulations between 1870 and 1890, coincident with the introduction of apochromatic lenses, mitochondria were observed and described by many authors under very diverse names. Unhappily, however, this group of

¹Contributions from the Anatomical Laboratory, Peking Union Medical College No. 1.

investigators failed to define them accurately and classified granules of totally different nature, under the same heading, with the true mitochondria.

Chief among them was Flemming (1882, p. 77), who studied granules and filaments in cells of many varieties in great detail, and, on the basis of his observations, erected his celebrated "filar" theory of the constitution of protoplasm. We now recognize among his "fila" our mitochondria of to-day, as well as other structures of totally different character. Unfortunately, Flemming's work was limited by the use of a mixture for fixation which contained a relatively large amount of acetic acid, which dissolved the mitochondria in many of the cells which he studied.

Altmann (1890, p. 100) was able to go considerably further than Flemming by the discovery of a much superior fixative containing no acetic acid and capable of preserving all the mitochondria. Unhappily, however, his technique was still far from specific and brought to light many granulations other than mitochondria, like zymogen and fat, which he included with them under the general heading of "Bioblasts." These "Bioblasts" he believed to be ultimate living particles, or elementary organisms, existing in the form of colonies in all cells, and he can not much be blamed for this mistake in view of the very real similarity between mitochondria and bacteria. Nevertheless his theories deterred many from the study of mitochondria.

F. and R. Zoja (1891, p. 237), following Maggi (1878, p. 326), made an elaborate study of mitochondria under the heading of "Plastiduli fuesinofili" and arrived at the interesting conclusion that they play a part in nutrition which approximates surprisingly closely to our modern ideas. Their results are of special value, inasmuch as they paid particular attention to invertebrates, while Altmann confined his observations to the cells of vertebrates. Others, about the same time, described mitochondria under the headings "Cytomicrosomes," "Neurosomes," "Plasmosomes," "Plasmafäden," and so on (see table 1).

Another reason for the lack of interest shown in mitochondria during the succeeding ten years is shown by a consideration of the technique. Virchow and his followers in pathological cytology directed their chief attention toward the nucleus; and biologists, dominated by the heredity problem, all looked in the same direction. Consequently their aim in making up fixatives was to show nuclear detail. For this purpose mixtures containing sublimate, alcohol, chloroform, or acetic acid were employed because of their rapid penetration and their action on chromatin. Now, these substances, unless certain precautions are taken, destroy mitochondria; so that the more attention was focussed upon the nucleus, the less chance there was for observation of mitochondria. Thus a vicious cycle was produced and maintained until fixatives made up with a basis of formalin, bichromate, or osmic acid were introduced.

The newer work on mitochondria may be said to begin with Benda's (1899*a*, p. 397) study of them in spermatogenesis. He modified Flemming's fluid by reducing the amount of acetic acid in it and devised a staining method by which the mitochondria may be colored with crystal violet, which, though not specific, has been of the utmost service to investigators. Benda also introduced the term "mitochondria."

In a review of this sort, one can not help being impressed with the existence of certain definite landmarks which have determined the whole trend of subsequent investigation and about which our ideas revolve. It can not be denied, for instance, that Meves's generalizations and theories, unjustified though they may be, have exercised a most stimulating effect upon the study of mitochondria. His statement (1908, p. 845), prompted by the discovery of mitochondria in all embryonic tissues, that all cellular differentiations are formed from mitochondrial coming at a time when the origin of these differentiations had been more or less explained to the satisfaction of cytologists without reference to mitochondria, attracted world-wide attention. Much work was hastily done anew with the most conflicting results, and even now uncertainty prevails in all branches of histogenesis. Similarly, his doctrine that mitochondria constitute in part the material basis of heredity, supported by the discovery that they enter the egg on fertilization, coming just when the chromatin hypothesis was receiving its strongest support at the hands of Morgan and others, could not fail to attract attention.

We owe much to Regaud (1908*d*, p. 720) and his school for supplying, through skillful indirect methods, the first accurate information regarding the chemical constitution of mitochondria, according to which they are made up of a combination of phospholipin and albumin. This immediately clarified our ideas, enabled us for the first time to form some estimate of their potentialities, and served as a point of departure for many investigations of value. It is important also to note that Regaud has thus brought the whole work on mitochondria into line with the recent tendency among physiological chemists and pathologists to become interested in the phospholipins, whereas formerly their whole attention was devoted to the study of proteins, being dominated by the tremendous impetus of Emil Fischer's work on protein synthesis, which attracted world-wide notice because of the psychological factor involved in the supposed manufacture of living substance.

The introduction of the dye janus green opened up a new and most valuable method of approach by making the study of mitochondria, specifically stained in living cells, so simple and satisfactory. It afforded an excellent basis for experimentation, dispelled any lurking doubt of the existence of mitochondria in the living condition, and supplied a means of detecting the artifacts produced by the older methods of fixation and staining.

The adaptation of methods of tissue culture by Champy (1912, p. 987; 1913*b*, p. 188) and the Lewises (1914, p. 330) to the study of mitochondria bids fair, if properly controlled, to give information of a type which can be obtained in no other way. It makes possible the continuous study with the microscope of vital processes going on in the cell. The Lewises in particular have devised methods by which they are able to keep selected mitochondria under observation for comparatively long periods of time and to see just what they do. Yet the method has its obvious limitations. In order to be most effective it should be used in conjunction with the methods of cell dissection recommended by Kite and Chambers.

And finally, the importance of the recognition of the sensitivity of mitochondria to pathological change becomes quite apparent when we remember that hereto-

fore the activities of cells in the investigation of disease and in the study of pathological processes have been gaged almost exclusively by the appearance of their nuclei. Now we have at our disposal another criterion of cell activity and of cell injury, the mitochondria, which we have already found to be of great and surpassing delicacy, and which respond, even before the nucleus, to injurious influences. Furthermore, this indicator is cytoplasmic, and as the cytoplasm is more intimately related to the environment than the nucleus, its study may yield very valuable information. It is an entirely different kind of indicator from the nucleus and we may confidently look to it to disclose facts which would never have been revealed by the study of the nucleus alone. That mitochondria are destined to play an important and conspicuous rôle in medical research, from now on, is quite apparent from Goetsch's (1916, p. 132) recent work on toxic adenomata of the thyroid gland. It may be said, by way of explanation, that all the work on goiter in time past has been vitiated, at the outset, by the fact that we lack a reliable criterion for the activity of the gland. It has come to be an embarrassing question to ask one to pick out from a number of thyroids, on the basis of the histological appearance, the one which was associated with the clinical symptoms of hyperthyroidism. The height of the epithelium, the appearance of the nuclei, and the amount of colloid are, at best, but poor aids in the dilemma. Now Goetsch has discovered that, in the cases which he has observed, the mitochondria are enormously increased in number where there are symptoms of hyperthyroidism. In other words, he has succeeded in correlating the perplexing clinical symptoms in the little-known condition of exophthalmic goiter, or Basedow's disease, with a definite anatomical change in the gland itself (*i. e.*, in the mitochondria). Many other conditions lend themselves to work along these lines and the outlook is promising.

TABLE 1.—*The Terminology of Mitochondria.*

Term.	Author.	Remarks.
Apparato reticolare interno.....	Golgi (1898, p. 64).....	Wrongly confused with mitochondria.
Archoplasm.....	Boveri (1888, p. 746).....	(ἀρχων, a ruler.) Material forming attraction sphere, astral rays, and spindle fibers. Wrongly confused with mitochondria.
Archoplasma-schleifen.....	Hermann (1891, p. 586).....	Same as mitochondria (Heidenhain, 1900, p. 527).
Basal filaments.....	Solger (1896, p. 248).....	Produced by the action of the fixative upon homogeneous basophilic material. They are not mitochondria (Bensley, 1911, p. 362).
Bâtonnets (Stäbchen).....	Heidenhain (1874, p. 47).....	Products of the transformation of mitochondria (Policard, 1912c, p. 458); see, however, Champy (1914, p. 380).
Binnennetz.....	Kopsch (1902, p. 934).....	Probably same as apparato reticolare interno.
Bioblasts.....	Altmann.....	(βίος, life, and βλαστός, a grain.) A heterogeneous class of granulation, partly composed of mitochondria.
Blepharoplastes.....		Analogous with mitochondria according to Perroncito (1910, p. 309).
Canalicular apparatus.....	Bensley (1910b, p. 179).....	Possibly same as the apparato reticolare interno.
Caryosomochondria.....	Arndt (1914, p. 55).....	By definition mitochondria arising from the caryosome.
Caulal chymoplasm.....	Conklin (1905, p. 218).....	An "organ-forming" substance containing mitochondria among other things.
Centrophormien.....	Ballowitz.....	Possibly the same as the nebenkern.
Chondriokonten.....	Meves (1907a, p. 401).....	Rod-like mitochondria.
Chondriolysis.....	Romeis (1912, p. 139).....	Solution of mitochondria.

TABLE I—Continued.

Term.	Author.	Remarks.
Chondriom	Meves (1907a, p. 403)	Cytoplasmic content of mitochondria.
Chondriomiten	Benda (1899a, p. 382)	Thread-like mitochondria.
Chondriomtom	Benda (1898, p. 397)	A feltwork of mitochondria.
Chondrioplastes	Champy (1913a, p. 157)	By definition plast-forming mitochondria.
Chondriorhâbden	Benda ¹	Shaft-like mitochondria.
Chondriosomen	Benda ¹	A generic term to include mitochondria of all forms.
Chondriosphären	Benda ¹	Sphere-like mitochondria.
Chondriotaxie.	Giglio-Tos and Granata (1908, p. 14)	The arrangement of granular mitochondria in threads.
Chordaneuroplasm	Conklin (1905, p. 218)	"Organ-forming" material which gives rise to chorda and nervous system; it naturally contains mitochondria.
Chromidial substance	Goldschmidt (1904, p. 124)	A basophilic, iron-containing substance, possibly of nuclear origin and quite distinct from mitochondria.
Chromochondries	Asvadourova (1913, p. 293)	By definition pigment-forming mitochondria.
Chymoplasm	Conklin (1905, p. 218)	"Organ-forming" material containing of course mitochondria.
Conduits de Golgi-Holmgren	Cajal (1908, p. 123)	Probably in part the same as the apparatus reticolare interno.
Cytoplasmic microsomes	Strasburger (1882, p. 479)	Cytoplasmic microsomes as contrasted with nuclear ones. The term obviously includes mitochondria.
Deutoplasm (deuteroplasm)	Van Beneden	Lifeless cytoplasmic constituents.
Dyctyosomes	Perroncito (1910, p. 315)	Produced by transformation of mitochondria (Fauré-Fremiet, 1913, p. 520).
Ectoplasm		(1) Peripheral cytoplasm often devoid of visible granulations; (2) an "organ-forming" substance (Conklin, 1905, p. 218).
Euchylemma	Butschli	More fluid part of protoplasm (Conklin, 1917, p. 357), contains mitochondria.
Endoplasm		(1) Deeper protoplasm often said to be nutritive by contrast (Renaud and Dubreuil, 1906b, p. 229); (2) an "organ-forming" substance (Conklin, 1905, p. 218).
Ergastoplasm	Garnier	Possibly similar to chromidial substance.
Ergastidions	Laguesse (1911, p. 276)	Introduced to indicate supposed relation of mitochondria to ergastoplasm; see, however, Regaud and Mawas (1909a, p. 462; 1909b, p. 229).
Fila	Flemming	Includes mitochondria as well as other structures.
Filaments of Herxheimer	Herxheimer	Occur in the germinative layer of the epidermis and are, according to Favre and Regaud (1910, p. 1138), true mitochondria.
Formations juxtannucleaires		Are in part mitochondria according to Perroncito (1910, p. 308).
Fuchsinophile granules	Alzheimer	These are mitochondria (Biondi, 1915, p. 227); nevertheless the term has been applied to many structures which are certainly not mitochondria.
Hyaloplasm	Hanstein	The clear homogeneous ground substance. It does not include mitochondria which may be separated out by centrifuging.
Idiozome	Meves (1897, p. 315)	(1) Same as nebenkern (Lewis and Lewis, 1915, p. 349); see, however, Duesberg (1910, p. 653); (2) same as nucleus of Balbiani (Sjovall, 1906, p. 577). Should not be confused with "idiosome," the hypothetical carrier of heredity.
Interstitialkôrner	Koelliker	The term has been applied to mitochondria as well as to other granulations in muscle-cells.
Intracellular bodies	Eberth	A product of the transformation of mitochondria according to Saguchi (1913, p. 177).
Karyochondria	Wildman (1913, p. 428)	Erroneously introduced to designate nuclear origin of mitochondria.
Kinetoplasm		Any energy-producing material.
Kinoplasm	Strasburger	(1) Same as chondriomtom, Prenant (1899, p. 429); (2) more solid part of protoplasm, Conklin (1917, p. 357).
Kôrnern	Brunn	A general term, but those described by him in spermatogenesis are true mitochondria.
Krystalplastiden	Wigand	Relation to mitochondria uncertain.
Metaplasm	Hanstein	Lifeless cytoplasmic material.
Microsomes	Hanstein	(μικρός, small, and σώμα, a body.) A most general term, including of course mitochondria.
Mitochondria	Benda (1898, p. 397; 1899a, p. 382).	(μίτρος, a thread, and χόσμος, a grain.) Fadenkôrnern, thread granules.

TABLE I—Continued.

Term.	Author.	Remarks.
Mitochondrial apparatus	Duesberg (1907, p. 284)	A general term, same as chondriom (Duesberg, 1908, p. 261).
Mitogels	Koltzoff (1906, p. 468)	Large mitochondrial masses formed by confluence.
Mitome	Flemming (1882, p. 77)	Same as hyaloplasm, according to Fauré-Fremiet (1912a, p. 408).
Mitosols	Koltzoff (1906, p. 468)	(Mitochondrosols) by definition, mitochondrial droplets.
Mitosoma	Platner (1889, p. 718)	Said to be the same as the nebenkern.
Myochondria	Jordan and Ferguson (1916, p. 94)	Introduced to designate mitochondria, which were thought to give rise to myofibrils.
Myoplasm	Conklin (1905, p. 218)	The "organ-forming" material giving rise to muscle, which of course contains mitochondria.
Nebenkern	Butschli	Same as micronuclei and geschlechtkerne (Hertwig, 1893, p. 212); probably a mitochondrial product.
Nebenkörper	Hermann	It is, according to Hermann, quite different from the nebenkern; the relation to mitochondria is uncertain.
Nematoblasten (Nematoplasten)	Zimmermann (1893, p. 215)	Some descriptions under this term apparently relate to mitochondria, while others do not.
Neurosome	Held (1895, p. 396)	Some of Held's neurosomes are mitochondria, others are not (Cowdry, 1913a, p. 487).
Nissl substance	Nissl	Same as chromidal substance (chromophile and tigroid substances, etc.), but quite distinct from mitochondria (Cowdry, 1911, p. 753; 1913b, p. 311).
Parabasal bodies		These, according to Alexieff (1917b, p. 501), are homologous with mitochondria.
Paramitome	Flemming (1882, p. 77)	(1) Same as paraplast (Fauré-Fremiet, 1912a, p. 408); (2) the more fluid part of protoplasm (Conklin, 1917, p. 357).
Paranucleus Péricaryonème (périnème)	Renaut and Dubreuil (1906b, p. 230)	Same as chondriomitom (Schäffer, 1912, p. 24). Mitochondria as they occur in certain connective-tissue cells.
Plasmosomes	Arnold (1907, p. 640; 1913, p. 433)	Mitochondrial nature doubtful (Duesberg, 1912, p. 823).
Plasomes	Wiensner	(πλάσμα, a thing formed, and σώμα, a body.) Ultimate vital units not to be confused with plastosomes.
Plastidulen	Maggi	Probably in part mitochondria.
Plastoehondrien	Meves (1910a, p. 150)	Granular mitochondria playing a designated part in histogenesis.
Plastoehondriomiten	Meves (1910a, p. 150)	Rows of granules.
Plastomonten	Meves (1910a, p. 150)	Long rods.
Plastolysis	Ciaccio (1913a, p. 725)	Loss of mitochondria.
Plastorhexis	Ciaccio (1913a, p. 725)	Breaking up of mitochondria into granules and vesicles.
Plastosomen	Meves (1910a, p. 150)	General term to include mitochondria of all shapes.
Praplastorhexis	Ciaccio (1913a, p. 725)	Pathological changes in mitochondria preliminary to plastorhexis.
Protoplasme supérieure	Prenant (1899, p. 421; 1910, p. 219)	A general term including archoplasm, kinoplasm, and ergastoplasm; does not relate to mitochondria.
Pseudochromosomes	Heidenhain (1900, p. 520)	Structures thought by him to be identical with mitochondria.
Saftkanälehen	Holmgren (1899, p. 139; 1903, p. 9)	Similar to apparato reticolare interno in some respects.
Sarcoplasmakörner		These are true mitochondria (Duesberg, 1910, p. 650).
Spheroplastes		Same as mitochondria (Fauré-Fremiet, 1907, p. 524).
Spiromes	Nelis (1899, p. 102)	Same as canalicular apparatus.
Substanz A	Cesaris-Demel (1907, p. 11)	Relation to mitochondria doubtful.
Tigroid substance		Same as Nissl substance.
Trophoplasm	Strasburger	(1) Nutritive cytoplasm (Renaut and Dubreuil, 1906b, p. 229); (2) more fluid cytoplasm (Conklin, 1917, p. 357); it contains mitochondria among other things.
Troples-pongium	Holmgren (1903, p. 9)	By definition, juice canals which penetrate cell from the exterior, evidently not synonymous with apparato reticolare interno and quite distinct from mitochondria.
Vegetative filaments	Altmann	Certain types of bioblasts occurring in gland-cells and identical with mitochondria.
Vernicles	Laguesse (1900, p. 5)	Certain filamentous mitochondria present in gland cells.
Vibrifiles	Swingle (1898, p. 110)	Relation to mitochondria doubtful.
Zentralkapsel	Heidenhain	Same as Binnennetz (Sjovall, 1906, p. 561).

II. NOMENCLATURE.

The terminology of mitochondria is unnecessarily complicated and confusing. The confusion has resulted from incoördination and from hasty individual action in elaborating new names, often only to discard them in a new paper in favor of some other. Some have attempted to convey information with respect to the morphology of mitochondria, others with regard to their physiology, and still others with respect to their colloidal chemistry. There has been no attempt to come together in a friendly spirit and arrive at some agreement or compromise. It is to be deplored that cytology should be so far behind gross anatomy in possessing no official list of terms like those of the Basle Anatomical Nomenclature. That the need for this is great may be seen by reference to table 1 (pp. 44-46).

The term "mitochondria" originated with Benda (1899*a*, p. 397), who introduced it to designate certain "Fädenkörnern" which he had been studying in spermatogenesis. It is derived from the Greek *μίτρος*, a thread, and *χόνδριος*, a grain.

It was soon discovered that mitochondria do not always occur in the form of thread-granules and investigators grasped at the idea that they should be named on the basis of their morphology. It was felt that the word "mitochondria" should be applied only in the sense in which Benda originally used it. Accordingly Meves (1907*a*, p. 401) devised the term "Chondriokont" to describe the rod-like forms and Benda,¹ almost simultaneously, came out with a whole list of terms with which to describe the various forms of mitochondria: "Chondriomiten" (threads), "Chondriorhäden" (shaft-like forms), "Chondriosphären" (spheres), and "Chondriom" or "Mitochondriom" (the cytoplasmic content of mitochondria of whatever form).

The words "chondriorhäden" and "chondriosphären" were not favorably received and were soon forgotten and investigators gradually drifted into the use of the following nomenclature for mitochondria based purely on morphology: "chondriosomes" (a generic term to include all forms); "mitochondries" (granules); "chondriocotes" (straight or curved threads); "chondriomites" (filaments of granules); and "chondriome" (the cytoplasmic content of chondriosomes). But, since all these forms grade by imperceptible transitions into each other, there was much confusion, and it is a difficult matter to find two investigators who are in entire agreement on the question of nomenclature.

As I have pointed out elsewhere (Cowdry, 1916*a*, p. 424), this system of terminology based on morphology is entirely superfluous in the light of recent work. We are coming to realize that the fundamental thing is the nature of the material rather than the form which it assumes (see p. 66). The Lewises (1915, p. 353), in the living cells of tissue cultures, were actually able to observe that mitochondria are continually changing in shape by bending in various directions, by shortening and thickening, by elongating, and by thinning, etc. They saw rods and threads change into granules, threads fuse to form networks, and many other alterations in the morphology of mitochondria. In other words, the same material under different conditions assumed different forms, as one would naturally expect.

¹In the discussion of a paper by Van der Stricht (1904, p. 145).

Now, the advocates of the above-mentioned terminology believe that it is of use because they think it convenient to have a special word to designate granular, rod-like, and filamentous forms, and rows of granules; but this system of terminology is not only cumbersome, confusing, and arbitrary, but it is also inadequate. That it is cumbersome needs no explanation. It is confusing because individual investigators do not all use it in the same way (see table 2). Indeed, it is not an easy matter to find two workers in agreement about it. It is arbitrary because it leads one to make sharp and clear-cut distinctions between different forms of mitochondria where none exist. Instead of saying, for instance, that the mitochondria in a cell are characterized by their great diversity of form, we must remark that chondriosomes of this particular cell occur in the form of mitochondria, chondriocotes, and chondriomites, and we are obliged to speak of the cellular content of chondriosomes as the chondriome. It is also inadequate because branching mitochondria—networks and circles and bleb-like swellings and many other varieties—also occur which are not provided for. Let us hope that no one will coin special names for them.

TABLE 2.

Term.	Dubreuil (1912, p. 127).	G. Arnold (1912b, p. 255).	Benda (1914, p. 21).	Duesberg (1917, p. 469).
Mitochondria.....	Isolated grains.....	Granules.....	General term.....	Granules.
Chondriocotes.....	Rods.....	Filaments.....	Not used.....	Filaments.
Chondriomites.....	Filaments of grains.....	Not used.....	Homogeneous filament	Not used.
Chondriosomes.....	More or less voluminous body, larger than pre- ceding, of irregular form.	Cellular content.....	General term.....	General term.
Chondriome.....	Cellular content.....	Chondriosome (p. 253).	Cellular content.....	Not used.

But the most pernicious systems of nomenclature arose several years later, when investigators became deluded into thinking that they knew something about the functional significance of mitochondria.

Koltzoff (1906, p. 468) has called the mitochondrial droplets "mitochondrosols" (or, briefly, "mitosols"), and the larger masses which he believes they form by confluence in the course of spermatogenesis he terms "mitogels."

The plastochondrial nomenclature of Meves is important in this connection. It is based upon his theory (Meves, 1908, p. 845) that, with the specialization of the embryo into different organs and tissues, primitively similar cells assume special functions which find expression in characteristic structures or differentiations. All these products, no matter how heterogeneous they may be, arise through the metamorphosis of one and the same elementary plasma constituent, the chondriosomes. He writes, subsequently (1910*a*, p. 150), that when we consider the important rôle that chondriosomes play in histogenesis we can speak (instead of chondriosomes) of "Plastosomen" ("Plastochondrien," "Plastochondriomiten" or short chondriomites, and "Plastocontent"). This terminology was accepted by all except those who oppose, or are skeptical, of the theoretical considerations which inspired it.

Duesberg (1912, p. 598) first accepted this system of nomenclature and adopted it, but later (1915, p. 35) rejected it for several reasons, but particularly in order to

avoid any confusion between the term "plastosomes" and the "plasmosomes" of Arnold. It may also be remarked that there is a possibility of confusing the term "plastosome" with the "plasmosome," or plasma-staining nucleolus as contrasted with the karyosome, as well as the "plasomes," or elementary vital units of Weisner, and the word "chondriome" with "chondroma," a cartilaginous tumor.

Still another series of names has been advocated, on the basis of the plast-like function of mitochondria; for it is thought that the mitochondria are plast-formers in plants and pigment-formers in animals. This view, so far as the plants are concerned, has a good mass of evidence in support of it. We owe the term "chondrioplaste" to Champy (1913*a*, p. 157) and the term "chromochondries" to Prenant (Asvadourova, 1913, p. 293).

Reference may be made to the term "karyochondria," which Wildman (1913, p. 428) introduced to describe certain mitochondria which he believed to arise from the nucleus in spermatogenesis in *Ascaris*. Arndt (1914, p. 55) for the same reason devised the term "Caryosomochondrien."

Since it is difficult to bear in mind all these terms and their various shades of meaning, we must make a more or less arbitrary selection of the one which appears to be the least objectionable, for it would obviously be highly inadvisable to follow the precedent and invent a new term. Priority is almost impossible to establish. I have accordingly selected the word "mitochondria" for the following reasons: (1) Its introduction by Benda in 1899*a* (p. 397) marks the beginning of much of the recent work on the subject; (2) ever since it has been, with but few exceptions, in quite general use, especially in this country; (3) it is simple and does not commit the user to any interpretation whatsoever.

Duesberg (1917, p. 469) opposes this position, as stated in my paper (1916*a* p. 424), as follows:

"To this I take the liberty of making the following remarks: The term 'mitochondria,' in Cowdry's sense, is not in general use even in this country; if the nomenclature must be based on morphology, one should, to be logical, reject 'mitochondria' as a general term, for 'mitochondrium' means granule and can not in consequence mean filament. The analogy with the term 'cell' is not adequate, for, though this word is not appropriate, it is, however, generally adopted; and it should not be forgotten that the invitation to use a term in a different sense from its original meaning comes in this case precisely from those who want to use 'mitochondria' as a general expression."

In answer I venture to point out:

(1) In 47 out of the 55 or more papers published in this country during the last five years (1912-1917) the term "mitochondria" has been used in the general sense, that is to say, in 85 per cent.

(2) The word "mitochondria" was introduced by Benda (1898, p. 397) and is derived from the Greek *μίτος*, a thread, and *χόνδρος*, a grain. In order to interpret it as granules one must ignore the "mito." That mitochondria are not simply granules (as Duesberg asserts) is also made perfectly clear by Benda himself (1899*a*, p. 382), who refers to them continually as "*Fadenkörnern*." The term "mitochondrium" is the Latin singular of the Greek compound word. The correct Greek singular is *mitochondrion*.¹

¹I am indebted for this information to Professor Miller, of the Greek department, Johns Hopkins University.

(3) The objection to the analogy with the term "cell" is not valid, since the word "mitochondria" has been generally adopted in Europe as well as in this country.

(4) The invitation to use the "term in a different sense than its original meaning" comes in this case from Duesberg if he says that "mitochondrium" means "granule," for the reason aforesaid.

That the word "mitochondria" has been repeatedly used as a general expression, both intentionally and unintentionally, is shown by the following citations:

Benda (1914, p. 20) states:

"In gelungenen Präparaten nach meiner sowohl wie anderen Methoden erkennt man innerhalb der Zellen die Mitochondrien in sehr verschiedener Form, Menge und Anordnung. Als Grundform betrachte ich die runde Gestalt, die in allen Zellarten gelegentlich vorkommt, in den Geschlechtszellen und den ersten Furchungszellen aber bei weitem vorherrscht. An Stelle der runden Körner findet man auch längliche Körnchen oder kürzeste Stäbchen, die alle als gleichwertig mit den Rundkörnern zu betrachten sind. Besonders bei spärlicher Körnermenge finden sich die Körner bisweilen unregelmässig im Zelleib verteilt und auch bei sehr grossen Anhäufungen ist naturgemäss eine Anordnung schwer zu erkennen. Sonst ist aber das Charakteristische, was in ihrem Namen von mir ausgedrückt werden sollte, ihre Neigung zur fadenförmigen oder reihenförmigen Anordnung, wo sie wie Streptokokken erscheinen. Man findet sie ferner in mehr homogenen gewundenen Fäden, die gewöhnlich unregelmässig segmentiert erscheinen und weiter als starre homogene oder segmentierte Stäbchen, endlich als grössere homogene Kugeln."

Favre and Regaud (1910, p. 1137) state:

"Les mitochondries (Benda) sont des organites du protoplasma qui, au contraire de l'ergastoplasma rare et contingent, ont une existence absolument générale dans les cellules. Ces organites se présentent tantôt sous la forme de granulations non ordonnées, tantôt sous la forme de granulations alignées en séries (on les appelle alors chondriomites), tantôt sous la forme de filaments continus (on les appelle alors chondriocentes), tantôt enfin (mais plus rarement) sous forme de corps volumineux de forme quelconque."

Duesberg (1907, p. 284) says:

"Unter obigem Titel sollen einige Mitochondrienstudien veröffentlicht werden. Ich habe mit Absicht diesen allgemeinen Ausdruck 'Mitochondrialapparat' gewählt, weil dieser über die Form dieser Gebilde keinen Vorbehalt einschliesst."

I am merely conservative in this and advocate nothing new. It may be objected that the old word "mitochondria" does not adequately describe the granulations as we now know them. This, to my mind, is only a very encouraging indication of the healthy condition of our science. Herein lies the key to the whole situation, for investigators have attempted to make the terminology keep pace with the discoveries, by making it convey information of all kinds. If a similar policy had been pursued with respect to the nucleus, or the cell itself, the results would have been even more disastrous. The question of nomenclature is but a very small part of the real problem.

III. LITERATURE.

VARIETIES OF CELLS.

For convenience I have reviewed the descriptive work on mitochondria in the different types of cells of the body (with special reference to the condition in man, given in *italics*) as well as in the tissues of higher plants. I have usually noted the most recent account, as well as the original reference, because experience has shown that the most up-to-date descriptions are often of far greater value than the old (and often vague) original records. Special care has been taken with the original descriptions because they are very difficult to trace accurately by reason of the fact that in them the mitochondria are invariably alluded to in a vague way under misleading terms (for which see p. 44). Such a summary serves as a kind of balance-sheet, and a glance at it will be sufficient to reveal the most conspicuous gaps which still persist in our knowledge of mitochondria and which might otherwise easily be overlooked (see also p. 144).

ANIMALS.

- Epithelial tissue:
 Epidermis, Favre and Regaud (1910, p. 1138).
 Hair follicles, Branca (1911, p. 559).
 Meibomian glands, Altmann (1894).
 Sebaceous glands, Altmann (1894); *Nicolas; Regaud, and Favre (1912a, p. 201).*
 Sweat glands, *Nicolas, Regaud, and Favre (1912b, p. 191).*
 Ceruminous glands, ———.
 Mammary glands, Altmann (1894, p. 159); Hoven (1911, p. 321).
 Glands of Moll, ———.
 Lachrymal glands, Altmann (1894, p. 159); Sundwall (1916, p. 202).
 Oil glands of birds, Altmann (1894, p. 159).
 Feathers, ———.
 Harder's gland, Altmann (1894, p. 159).
 Leydig's "Schleimzellen," Meves (1907b, p. 565).
 Teeth, Manca (1913, p. 121).
- Alimentary tract:
 Hypobranchial gland, Grynfeltt (1912a, p. 12).
 Parotid gland, Altmann (1894, p. 159).
 Ebner's gland, ———.
 Submaxillary gland, *Regaud and Mavras (1906b, p. 225).*
 Blandin's gland, ———.
 Tonsil, *Alagna (1911, p. 27).*
 Labial (buccal, molar) glands, ———.
 Retrolingual gland, ———.
 Esophagus, Kollmann and Papin (1914, p. 222).
 Principal and accessory labial glands, *Fauré-Fremiet (1910c, p. 3).*
- Stomach:
 Parietal cells, Eklöf (1914, p. 224). See, however, *Regaud (1908a, p. 18).*
 Chief cells, Altmann (1894, p. 157); Eklöf (1914, p. 223).
 Goblet cells, Eklöf (1914, p. 227).
 Brunner's glands, Eklöf (1914, p. 225).
 Paneth cells, Eklöf (1914, p. 225).
- Pancreas:
 Acinus cells, *Altmann (1894, p. 158).*
 Islet cells, *Bensley (1911, p. 368).*
 Hepato-pancreas, *Guéysson-Péllissier (1910, p. 18).*
- Alimentary tract—*continued.*
 Liver, Altmann (1894, p. 155).
 Bile ducts, *Pollicard (1914, p. 623).*
- Respiratory tract:
 Bowman's lungs, ———.
 Trachea and lung, *Prenant (1911b, p. 337); Meves and Tsukaguchi (1914, p. 289).*
- Urino-genital tract:
 Kidney (metanephros), *Heidenhain (1874, p. 47); Benda (1899b, p. 331); Regaud (1908a, p. 15); Pollicard (1915, p. 539).*
 Pronephros and mesonephros in amphibia, *Luna (1913f, p. 131).*
 Bladder, ———.
 Urethra, ———.
 Glands of Littre, ———.
 Spermatogenesis, v. *Brunn (1884, p. 132)* under heading of "Körner."
 Interstitial cells, *Benda (1899b, p. 351).*
 Epididymis, *Fuchs (1901, p. 328).*
 Cowper's glands, ———.
 Seminal vesicles, *Akatsu (1903, p. 566).*
 Prostate, *Akatsu (1903, p. 566); Dominici (1913 p. 295).*
 Prepuccial glands (glands of Tyson), *Altmann (1890, p. 99).*
 Corpus cavernosum, ———.
 Corpus spongiosum, ———.
 Ovary, *Zoja brothers (1891, p. 259)*, under head of "Plastidulen."
 Interstitial cells of ovary, *d'Athias (1912, p. 448).*
 Egg cells, *Van der Stricht (1905, p. 7).*
 Lutein cells, *Levi (1913, p. 526); Corner (1914, p. 76).*
 Fallopian-tube glands, *Altmann (1894, p. 159).*
 Uterus, *Romeis (1913a, p. 9).*
 Vagina, ———.
 Clitoris glands, *Altmann (1890, p. 99).*
 Bartholin's glands, ———.
 Placenta, cells of *Langhans, Van Cauwenberghe, confirmed by de Kervily (1916, p. 589).*
 Coecygeal gland, *Altmann (1890, p. 145).*

ANIMALS—Continued.

- Connective tissue:
 Fibroblasts, Dubreuil (1913, p. 95).
 Clasmatoocytes, Dubreuil (1913, p. 87).
 Plasma cells, *Schridde* (1905, p. 729); Dubreuil (1909, p. 80); and *Dubreuil and Favre* (1914b, p. 24).
 Mast cells (basophilic cells), mitochondria are absent according to Galeotti (1895, fig. 18). (I have also found that they are absent in mast cells occurring in the thymus, but present in those in bone marrow.)
 Cartilage cells, Flemming (1882, p. 22); *Dubreuil* (1911a, p. 139); *Pensa* (1913b, p. 557).
 Bone cells, Dubreuil (1910b, p. 1101).
 Osteoblasts, Dubreuil (1910b, p. 1101; 1913, p. 119).
 Osteoblasts (myeloplaxs, giant multinucleated cells etc.), cells of Bizzozero, Dubreuil (1910a, p. 72; 1913, p. 132).
 Odontoblasts, Prenant (1911b, p. 335).
 Fat cells, Metzner (1890, p. 82); Dubreuil (1913, p. 98).
 Muscular tissue:
 Striated, Benda (1899a, p. 379); Regaud and Favre (1909, p. 298).
 Smooth, Benda (1899a, p. 379).
 Cardiac, Regaud (1909b, p. 426).
 Purkinje, Mironesco (1912, p. 30).
 Nervous tissue:
 Neuroglia cells, Nageotte (1909, p. 826), *Collin* (1913a, p. 179).
 Sheath cells, ———.
 Nerve cells, Altman (1890, p. 52); *Cowdry* (1914a, p. 11).
 Ear, organ of Corti, Van der Stricht (1918, p. 63).
 Eye:
 Retina, Leboucq (1909, p. 576); *Collin* (1913b, p. 1358).
 Lens, Maggiore (1912, p. 118).
 Cornea, Prenant (1911b, p. 333).
 Nose:
 Nasal mucous membrane, ———.
 Olfactory bulbs, ———.
 Organ of Jacobson, ———.
 Motor end plate, ———.
 Meissner's corpuscle, ———.
 Pacinian corpuscles, ———.
 Crandy's corpuscle, ———.
 Herbst's corpuscle, ———.
 Taste buds, ———.
 Endothelium:
 Artery, mitochondria frequently described incidentally.
 Vein, mitochondria frequently described incidentally.
 Lymphatic, ———.
- Reproductive system:
 Thallophytes:
 Unicellular forms: Saccharomyces. Endomyces. *Guilliermond* (1914b, fig. 10).
 Conidia conidiophores: *Penicillium*, *Guilliermond* (1914b, fig. 10); *Albugo*, *Lewitsky* (1913, pl. 21).
 Oospore oogonium, oogonium anlage; *Albugo*, *Lewitsky* (1913, pl. 21).
 Zygospore, gametangia, suspensors: *Sporodinea*, *Moreau* (1915a, p. 171).
 Ascospore, asci, ascogenous hyphae, paraphyses; *Pustularia*, *Peziza*, *Aleuria*, *Guilliermond* (1914b, figs. 12-15; 1913a, fig. A; 1913b, figs. 11-13).
 Basidiospore, basidia, mother cells of probasides, hymenial layer; *Psalliota*, *Puccinia*, *Beauverie* (1914a, p. 798; 1914b, p. 359).
 Teliospore, *Puccinia*, *Beauverie* (1914b, p. 359); *Phragmidium*, *Mme. Moreau* (1914, p. 421).
- Mesothelium:
 Peritoneum, ———.
 Pericardium, ———.
 Pleura, ———.
 Synovial membrane, ———.
 Plexus chorioideus, *Hworostuchin* (1911, p. 232).
 Blood:
 Erythrocyte, ———.
 Young erythrocytes, *Meves* (1907a, p. 402).
 Normoblasts, *Meves* (1907a, p. 402).
 Erythroblast, *Meves* (1911a, p. 495).
 Lymphocyte, *Schridde* (1905, fig. 69).
 Transitionals, *Naegeli* (1912, p. 175).
 Lymphoblast, *Schridde* (1907, p. 231).
 Polymorphonuclear neutrophile, *Benda* (1899a, p. 380); *Cowdry* (1914b, p. 278).
 Polymorphonuclear eosinophile, *Benda* (1899a, p. 380); *Cowdry* (1914b, p. 279).
 Polymorphonuclear basophile, ———.
 Neutrophile myelocyte, ———.
 Eosinophile, ———.
 Basophile, *Dubreuil* (1911a, p. 138).
 Myeloblast, *Schridde* (1907, p. 231); see, however, *Klein* (1910, p. 407).
 Megakaryocytes, *Prenant* (1911b, p. 335); see, also, *Dubreuil* (1910a, p. 73).
 Polykaryocytes, *Dubreuil* (1910a, p. 72).
 Platelets, *Cowdry* (1914b, p. 275).
 Macrophages, *Atagna* (1911, p. 37).
 Ductless glands:
 Thymus:
 Small cells and epithelial cells, *Salkind*.
 Hassal's corpuscles, ———.
 Thyroid, Galeotti, under the name of "fuchsinophile granules," see *Bensley* (1916, p. 41).
 Suprarenal gland, *Mulon* (1910a, p. 103), *da Costa* (1916, p. 164).
 Hypophysis, under heading of "Altmann's granules," *Prenant*, *Bouin*, and *Maillard* (1906, p. 52).
 Epiphysis, ———.
 Parathyroid, *Bobeau* (1911, p. 183).
 Carotid body, ———.
- Tissue cultures:
 Kidney, *Champy* (1914, p. 312).
 Thyroid, *Champy* (1912, p. 987; 1913b, p. 192; 1915, p. 63).
 Embryonic tissues, *Lewis* (1914, p. 330); *Levi* (1916a, p. 101); *Maximow* (1916b, p. 465).

PLANTS.

Reproductive system—continued.

Thallophytes—continued.

- Uredospore, *Coleosporium*, *Mme. Moreau* (1914, p. 421).
 Ecidiospore, *Phragmidium*, *Mme. Moreau* (1914, p. 421).
 Bryophytes and pteridophytes:
 Spore, sporogenous-tissue cells, tapetum, spore-mother cells; *Fumarica*, *Sapchin* (1915, pl. 22); *Anthoceras*, *Scherer* (1913, pl. 20).
 Sperm, spermogenous-tissue cells, sperm mother-cells, antheridium mother-cells; *Polytrichum*, *Fumarica*, *Bryum*, *Marchantia*, *Sapchin* (1915, pls. 22-26); *Anthoceras*, *Scherer* (1913, pl. 20).
 Egg, ventral-canal cell, neck-cells of archegonium; *Fumarica*, *Bryum*, *Sapchin* (1915, pl. 26); *Anthoceras*, *Scherer* (1913, pl. 20).

PLANTS—*Continued.*Reproductive system—*continued.*

- Spermatophytes:
 Pollen, pollen mother-cells, tapetum; Nymphaea, Meves (1904, p. 284); Asparagus, Lewitsky (1910, pl. 17); Lilium, Cucurbita, Guillaiermond (1912a, fig. 6; 1914b, fig. 6).
 Embryo sac, ovule, antipodals, synergids, embryo tegument, nutritive cells; Lilium, Guillaiermond (1912a, figs. 4, 5; 1914b, fig. 13); Pensa (1911, fig. 2).
 Seed, barley, Ricinus, Guillaiermond (1912a, fig. 7).
- Vegetative system:
 Filaments of algae and fungi; Vaucheria, Achlya, Rudolph (1912, pl. 18); Penicillium, Endomyces, Guillaiermond (1914b, fig. 10).
 Apical cells of algae, Cystoseira, Nicolosi-Roncati (1912b, p. 144); Fucus, Le Touze (1912, pl. 9).
 Primary meristem of root, dermatogen, periblem, plerome, root-cap; Asparagus, Lewitsky (1910, pl. 17); Trianea, Sapehin (1915, pl. 11).
 Primary meristem of stem-tip, buds, dermatogen, periblem, plerome; Elodea, Euphorbia, Sapehin (1915, pls. 10-11); Asparagus, Rudolph (1912, pl.

Vegetative system—*continued.*

- 18); Lewitsky (1910, pl. 17); Tropaeolum, Guillaiermond (1912a, fig. 2); Tulip, Pensa (1911, figs. 6, 7); Secondary meristem, cambium, Guillaiermond (1912a, p. 358).
 Epidermis of stem, root, leaf, flower, fruit; Asparagus, Trianea, Sapehin (1915, pls. 10, 11); Chenopodium, Juglans, Iris, Tulip, Clivia, Tropaeolum, Guillaiermond (1917, p. 233; 1914b, fig. 14).
 Mesophyll cells, palisade cells, leaves, carpels; Nereus, "haricot," Guillaiermond (1914b, fig. 11, 12; 1912a, pl. 18).
 Leaf cells, stem-tip of mosses, Polytrichum, Sapehin (1913, pl. 14).
 Leaf cells of ferns; Aspidium, Scolopendrium, Pensa (1911, figs. 3, 4, 5).
 Protonema cells of mosses, Funaria, Sapehin (1913, pl. 14).
 Secreting cells, Juglans, Guillaiermond (1914a, p. 566).
 Hair cells, Cucurbita, Maximow (1913, p. 243).
 Giant cells of Heterodera galls; Vitis, Némec (1910, p. 171).

DIFFERENT TYPES OF ORGANISMS.

It is often of interest also to know in just what forms, both animal and vegetable, mitochondria have been described and by whom. I have prepared the following summary with this end in view. The work of the older authors is very difficult to interpret, especially that of Retzius, for he has described structures derived from mitochondria in mature spermatozoa of hundreds of different forms. These bodies do not possess the staining reactions of mitochondria, though they are formed from them; they are not soluble in acetic acid and may be demonstrated by ordinary methods of technique. Strictly speaking, they are assuredly not mitochondria, but since they develop from mitochondria (see page 101), it is difficult to say when they cease to be mitochondria. For a description of them I venture to refer to the excellent account of Duesberg (1912, page 615).

ANIMALS.

Protozoa:

Rhizopoda:

- Amœba gorgonia (?), Fauré-Fremiet (1910a, p. 507).
 Cochliopodium pellucidum, Fauré-Fremiet (1910a, p. 508).
 Actinophrys sol, Fauré-Fremiet (1910a, p. 509).
 Amœba proteus, Vonwiller (1915, p. 485).
 Amœba limax, L. and R. Zoja (1891, p. 242).
 Amœba chondrophora, Arndt (1914, p. 51).

Mastigophora:

- Cryptomonas ———, Fauré-Fremiet (1910a, p. 510).
 Monadi, L. and R. Zoja (1891, p. 242).
 Chilomonas paramoecium, Prowazek, according to Fauré-Fremiet (1910a, p. 510).
 Trypanosoma lewisi, Shipley (1916, p. 444).
 Blastocystis enterocola, Trichomonas angusta, Alexeiff (1916b, p. 1074).
 Giardia cucicula, aradenalis, muris, and agilis, Trichomonas batrachiorum and muris, Hexamastix termitis, Octomastix parva and motella, Octomitus intestinalis, Trichonympha agilis, Entrichomastix motella, and Tetramastix, Alexeiff (1917b, p. 499).

Protozoa—*continued.*

Sporozoa:

- Monocystis ascidia, Hirschler (1914, p. 304).
 Hemogregarina sergentium, Viguier and Weber (1912, p. 92).

Infusoria:

- Vorticella, under heading of "Spheroblasts," by Czernak, according to Fauré-Fremiet (1910a, p. 478).
 Vorticella convallaria, Fauré-Fremiet (1910a, p. 523).
 Epistylis flavicans, under heading of "Spheroblasts," by Greef, according to Fauré-Fremiet (1910a, p. 478).
 Toxodes rostrum, Fauré-Fremiet (1910a, p. 495).
 Stentor, Nassula, Urostyla grandis, Fauré-Fremiet (1910a, p. 496).
 Glaucoma piriformis, Fauré-Fremiet (1910a, p. 501).
 Trichodinopsis paradoxa, Cyclostoma elegans, Fauré-Fremiet (1910a, p. 502).
 Carchesium polyipinum, Spirostomum. ambiguum, Fauré-Fremiet (1910a, p. 504).
 Campanella ———, Fauré-Fremiet (1910a, p. 506).
 Paramoecium caudatum, Fauré-Fremiet (1910a, p. 511).

ANIMALS—Continued.

Protozoa—continued.

Infusoria—continued.

- Trachelius ovum*, Fauré-Fremiet (1910a, p. 512).
Strobilidium gyrans, Fauré-Fremiet (1910a, p. 514).
Cyrtarocycelis Ehrenbergii, under heading of "Chromidia," Gesa Entz, jr., according to Fauré-Fremiet (1910a, p. 514).
Tintinnidium inguulinum, Fauré-Fremiet (1910a, p. 514).
Opisthnecta henneguyi, Fauré-Fremiet (1910a, p. 516).
Trichodinopsis paradoxa, Fauré-Fremiet (1910a, p. 517).
Opercularia racemosa, Fauré-Fremiet (1910a, p. 519).
Opercularia notonecta, Fauré-Fremiet (1910a, p. 520).
Noetiluca miliaris, Fauré-Fremiet (1910a, p. 524).
Opalina ranarum, Colopoda cucullus, *Stentor cucullus* and polymorphus, *Isotheria prostoma*, *Entodinium bursa* and *minus*, *Diplodinium maggii* and *cattanei*, *Butschlia parva*, L. and R. Zoja (1891, pp. 243, 244).
Blantidium enterozoön, Benda (1899b, p. 168).
Fabria salina, *Condylostoma patens*, *Epiclintes ambiguus*, *Strombidium sulcatum*, *Strombidium stylifer*, Fauré-Fremiet (1912a, p. 411).

Porifera:

- Spongilla fluviatilis*, L. and R. Zoja (1891, p. 244).

Cœlenterata:

Hydrozoa:

- Hydractinia echinita*, eggs, Beckwith (1914, p. 204).
Halistemma rubrum, *Forskalia contorta*, *Physophora hydrostatica*, *Gleba hippopus*, *Praya maxima*, spermatogenesis, under heading of "Cytomicrosomes," Pietet, according to Fauré-Fremiet (1910a, p. 571).
Hydra vulgaris, L. and R. Zoja (1891, p. 244).

Scyphozoa:

- Aurelia aurita*, Tsukaguchi (1914, p. 117).

Actinozoa: ———.

Ctenophora: ———.

Platyhelminthes:

Turbellaria:

- Plagiostoma girardi*, spermatids, Weygandt (1907, p. 271).
Planaria adherens, almost all cells of the body, Korotneff (1909, p. 1010).

Trematoda:

- Dicrocoelium lanceatum*, spermatogenesis, Dingler (1910, p. 698).

Cestoda: ———.

Nemertinea: ———.

Nemathelminthes:

Nematoda:

- Ascaris lumbricoides*, male and female germ-cells, Hirschler (1913, p. 353).
Ascaris megaloccephala, gonocytes, Fauré-Fremiet (1912b, p. 346); L. and R. Zoja (1891, p. 245).
Filaria papillosa, Meves (1915b, p. 38).

Acanthocephala:

- Chaetognatha*: ———.

Trochelminthes:

- Rotifera*: ———.

- Dinophleca*: ———.

- Gastrotricha*: ———.

Molluscoidea:

Polyzoa:

- Membranifera pilosa* L., spermatogenesis, Bonnevie (1907, p. 578).

Molluscoidea—continued.

- Phoronida*: ———.

- Brachiopoda*: ———.

Echinodermata:

Asteroidea:

- Asterias glacialis*, oocyte, Schaxel (1911, p. 346).
Asteracanthion tenuispinis, L. and R. Zoja (1891, p. 250).

Ophiuroidea: ———.

Echinoidea:

- Strongylocentrotus lividus*, *Arbacia pustulosa*, *Echinus microtuberculatus*, *Sphaerechinus granularis*, *Spatangus purpureus*, under heading of "Cytomicrosomes," in spermatogenesis, Pietet, according to Fauré-Fremiet (1910a, p. 571).

Holothuroidea: *Holothuria tubulosa* and *polii*, oocytes, Schaxel (1911, pp. 344, 347).Crinoidea: *Comatula mediterranea*, L. and R. Zoja (1891, p. 250).

Annulata:

Chaetopoda:

- Nereis virens*, nerve-cells, Cowdry (1914a, p. 11).
Serpula uncinata, *Nais proboscidea*, L. and R. Zoja (1891, p. 248).

- Tapinambis teginxin*, kidney, Mayer and Rathery (1909, p. 335).

- Lumbricus herculeus* and *terrestris*. *Allobophora terrestris*, kidney, Maziariski (1903).

- Aricia fœtida*, egg segmentation, Schaxel (1912, p. 402).

Myzostoinida: ———.

Gephyrea: ———.

Archi-annelida: ———.

Hirudinea:

- Hirudo medicinalis*, L. and R. Zoja (1891, p. 248).

Arthropoda:

Crustacea:

- Astacus fluviatilis*, spermatogonia, and spermatocytes, Prowazek (1902, p. 448); L. and R. Zoja (1891, p. 253).

- Homarus vulgaris*, egg-cells, under heading of "Pseudo-chromosomen," F. Henschen (1904, p. 16).

- Leander adpersus*, spermatogenesis, Spitschakoff (1909, p. 26).

- Menippe mercenaria*, spermatogenesis, Binford (1913, p. 156).

- Gammarus pulex*, spermatid, Köster (1910, p. 491).

- Callinectes hastatus*, *Cancer borealis*, *Homarus americanus*, nerve-cells, Cowdry (1914a, p. 11).
Eupagurus prideauxii and *Eriphia spinifrons*, under heading of "Microsomes," by Grobden, according to Fauré-Fremiet (1910a, p. 551).

- Maia squamada*, *Carcinus maenas*, and *Eupagurus bernhardus*, spermatogenesis, Labbé (1904, p. xi).

- Scyllarus arctus*, *Portunus corrugatus*, *Pagurus striatus*, and *Galathea squamifera*, spermatogenesis, Koltzoff (1906, p. 565).

- Galathea strigosa*, *Palinurus vulgaris*, and *Corrugus fortunatus*, hepato-pancreas, Guicysse-Pélessier (1910, p. 18).

- Pandarus sinuatus*, spermatogenesis, McClendon (1910, p. 234).

ANIMALS—Continued.

- Onychophora: ———.
- Myriopoda: ———.
- Pachyulius varius*, spermatocytes, Oettinger (1909, p. 593).
- Julus terrestris*, eggs, Fauré-Fremiet (1908, p. 1057).
- Lithobius forficatus* and *scolopendra*, spermatogenesis, Fauré-Fremiet (1910a, p. 582).
- Geophilus longicornis* and *carphophilus*, *Polyxenus lagurus*, *Polygonium germanicum*, *Blaniulus guttulatus*, oocytes, Fauré-Fremiet (1910a, p. 592).
- Arachnida:
- Limulus polyphemus*, eggs, Munson (1898, p. 111).
- Argas miniatus*, spermatogenesis, Casteel (1917, p. 650).
- Ixodes reduvius*, spermatocytes, Nordenskiöld (1909, p. 514).
- Tegenaria domestica*, egg-cells, under heading of Nucleus of Balbian, Van der Stricht (1898, p. 135); L. and R. Zoja (1891, p. 253).
- Euscorpis carpathicus* L., *Buthus eupeus*, spermatogenesis, Sokolov (1913, p. 395).
- "Arizona scorpion," Wilson (1916, p. 539).
- Insecta:
- Forficula auricularia*, Valette St. George (1887), *Blatta germanica*, *Periplaneta*, spermatids, Hennegy (1904).
- Pezotettix pedestris*, *Acridium ægyptium*, *Psophus stridulus*, *Decticus verrucosus*, *Locusta viridissima*, Buchner (1909, p. 361).
- Hydrophilus piceus*, L. and R. Zoja (1891, p. 274).
- Pamphagus marmoratus*, spermatogenesis, Gilio-Fos and Granata (1908, p. 115).
- Stenobothrus biguttulus*, spermatogenesis, Gerard (1909, p. 599).
- Locusta viridissima*, *Gryllus campestris*, spermatogenesis, Hennegy (1904).
- Gryllus assimilis*, spermatid, Baumgartner (1902, p. 50).
- Gryllus campestris*, primary spermatocytes, Hennegy (1904).
- Gryllotalpa vulgaris*, spermatogonia, Buchner (1909, p. 336); *Gryllotalpa borealis*, sex-cells, Payne (1916, p. 305).
- Smerinthus populi*, *Pieris brassicæ*, *Oxygia antiqua*, *Porthesia similis*, *Pieris rapæ*, *Spilosoma lubricipeda*, *Euchelia jacobæ*, *Cossus ligniperda*, *Bombyx lanestrus* and *rubi*, *Arbaxus grossularia*, sex-cells, Gatenby (1917, p. 413).
- Aphis saliceti*, spermatogenesis, v. Baehr (1909, p. 290).
- Pyrrhocoris apterus*, spermatozoa, Fauré-Fremiet (1910a, p. 548).
- Notonecta glauca*, spermatocytes, Pantel and de Sinéty (1906, p. 128).
- Notonecta insulata*, *irrorata*, and *undulata*, spermatogenesis, Browne (1913, p. 99).
- Hydrometra lacustris*, spermatogonia, Wilke (1907, p. 687).
- Euschistus*, spermatogenesis, Montgomery (1911, p. 776).
- Pygera bucephala*, spermatogenesis, Meves (1900, p. 556).
- Bombyx* ———, spermatogenesis, Hennegy (1904).
- Oryctes nasicornis*, spermatogenesis, Prowazek.¹
- Insecta—continued.
- Silpha carinata*, spermatogenesis, N. Holmgren (1902, p. 197).
- Cybister rosellii*, spermatocytes, Voinov (1903, p. 222).
- Dytiscus marginalis* and *circumcinctus*, spermatogonia, Schäfer (1907, p. 543).
- Apis mellifica*, spermatogenesis, Meves (1907c, p. 478).
- Vespa crabro* and *germanica*, Meves and Duesberg (1908, p. 581).
- Camponotus herculeanus*, spermatocytes, Läms (1908, p. 530).
- Libellula* ———, muscle, Holmgren (1909, p. 305).
- Chorthippus curtipennis*, spermatogenesis, Lewis and Robertson (1916, p. 113).
- Trichiosoma lucorum*, oogenesis, Govaerts (1913, p. 437).
- Leptinotarsa decemlineata*, oogenesis, Hegner (1914, p. 417).
- Mollusca:
- Pelecypoda:
- Venus mercenaria*, nerve-cells, Cowdry (1914a, p. 11).
- Anodonta cellensis*, muscle, Bruck (1914, p. 481).
- Amphineura: ———.
- Gastropoda:
- Enteroxenes östergeni*, spermatogenesis, Bonnevie (1904, p. 269).
- Conus mediterraneus*, *Vermetus gigas*, spermatogenesis, Kuschakewitsch (1911, p. 532).
- Fulgur canaliculatus*, nerve-cells, Cowdry (1914a, p. 11).
- Helix pomatia*, *Acanthopsale rubrovittata*, L. and R. Zoja (1891, pp. 251, 253).
- Murex medicinalis*, hypobranchial gland, Grynfeltt (1912b, p. 263).
- Helix hortensis*, *pomatia*, and *planorbis*, spermatogenesis, Benda (1899a, p. 377).
- Columbella rustica*, spermatogenesis, Schitz (1916, p. 45).
- Arion rufus*, spermatogenesis, Fauré-Fremiet (1910a, p. 631).
- Arion empiricorum*, egg-cells, Läms (1910).
- Cymbulia peronii*, spermatogenesis, under heading of "Microsomes," by Pietet, according to Fauré-Fremiet (1910a, p. 571).
- Cephalopoda:
- Octopus vulgaris*, kidney, under heading of "Enclaves intra-cellulaires," Mayer and Rathery (1907, p. 41).
- Loligo pealii*, nerve-cells, Cowdry (1914a, p. 11).
- Chordata:
- Adelochorda: ———.
- Urochorda: ———.
- Ciona intestinalis*, *Ascidia mentata*, *Molgula socialis*, *Cynthia tatraëdra*, *Cynthia morus*, eggs, Loyez (1909, p. 190); L. and R. Zoja (1891, p. 255).
- Cynthia partita*, embryos, Duesberg (1913, p. 463).
- Cynthia microcosmus*, oocytes, Bluntschli (1904, p. 427).
- Phallusia mammillata*, fertilization, Meves (1913, p. 215).
- Cyclostomata:
- Petromyzon marinus* and *Ammocoetes* branchials, nerve-cells, Mawas (1910a, p. 126).
- Myxine glutinosa*, primary spermatocytes, Schreiner A. and K. E. (1905, p. 296).

¹Arb. a. der. zool. Instit., Wien und Triest, 1902.

ANIMALS—Continued.

Chordata—continued.

Pisces:

Perca americana, Morone americana, *Salmo irideus*, *Fundulus heteroclitus*, embryos, Duesberg (1917, p. 465); *Coregonus murena*, germ-cells, Aunap (1913, p. 454); *Box salpa*, *Tinea vulgaris*, Corti (1913, p. 52); *Anguilla vulgaris*, interrenal, in testine, Conolly (1913, p. 408).

Bream, pike, and chevasson, kidney, Policard and Mawas (1906, p. 218).

Scyllium canalicula, choroid plexus, Grynfeldt and Euziere (1913b, p. 104).

Salmo ———, eggs, Cernak (1902, p. 159).

Amphibia:

Pleurodeles waltlii, pancreas, Chaves (1912, p. 30).

Molge waltlii, pancreas, Chaves (1915, p. 46).

Rana fusca, esculenta, arvalis, mugiens, spermatogenesis, Broman (1907, p. 348).

Rana viridis, kidney, Policard (1905, p. 381).

Rana temporaria, L. and R. Zoja (1891, p. 256).

Triton alpestris and *vulgaris*, intestine, Champy (1913a, p. 76).

Triton cristatus, L. and R. Zoja (1891, p. 256).

Bombinator igneus, intestine, Champy (1913a, p. 82).

Bufo vulgaris, kidney, Policard (1905, p. 381).

Necturus maculatus, *Rana palustris*, nerve-cells, Cowdry (1914a, p. 2).

Axolotl, *Bombinator igneus*, spermatogenesis, Champy (1913a, p. 64).

Tinea vulgaris, *Hyla arborea*, L. and R. Zoja (1891, p. 256).

Rhacophorus, larvae, Saguchi (1913, p. 185).

Geotriton fuscus, pancreas, Levi (1912, p. 579).

Reptilia:

Clemmys marmorata, *Lacerta muralis*, oögonia, Munson (1904, plate vii).

Tropidonotus viperinus, kidney, Regaud (1908a, p. 15).

Vipera latastei, pancreas, Chaves (1915, p. 46).

Pseudemys hieroglyphica, nerve-cells, Cowdry (1914a, p. 2).

Reptilia—continued.

Testudo graeca, nerve-cells, Busacca (1913, p. 327).

Cistudo europea, muscle, Aimé (1911, p. 270).

Platydaetylus muralis, *Lacerta viridis* and *muralis*, L. and R. Zoja (1891, p. 256).

Aves:

Hirundo communis, *Chelidon urbica*, *Passer domesticus*, *Fringilla*, *Turdus merula*, *Parus major*, *Regulus cristatus*, ovaries, Van Durme (1914, p. 78).

Gallus domesticus, embryos, Meves (1907a, p. 399).

Columba livia, nerve-cells, Cowdry (1912, p. 34).

Parus major, *Muscicapa grisola*, follicle cells, under heading of "Pseudochromosomes," D'Hollander (1902, p. 170).

Fringilla cannabina, oocytes, Dulzetto (1916, p. 217).

Sylvia atricapilla, *Gallus domesticus*, *Emberiza citrinella*, L. and R. Zoja (1891, p. 256).

Mammalia:

Erinaceus europaeus, intestine, Corti (1913, p. 188).

Didelphus virginiana, spermatogenesis, Jordan (1911, p. 58).

Phocena communis, under heading of "Körnerchen," Ballowitz (1907, p. 234).

Sus scrofa, blood-cells, Shipley (1915, p. 61).

Microtus subterraneus, pancreas, Chaves (1915, p. 46).

Canis familiaris, egg-cells, Van der Stricht (1908, p. 3).

Vesperugo noctula, egg-cells, under heading of "Pseudochromosomes," Van der Stricht (1902, p. 5).

Vespertilio murinus and *blasii*, *Rhinolophus euriale*, *Miniopterus schreiberi*, ovaries, Levi (1913, p. 518).

Many apes, spermatogenesis, Moreaux (1909, p. 371).

Macacaes rhesus, nerve-cells, Cowdry (1914a, p. 3).

Vespertilio serotinus, suprarenal, Da Costa (1913, p. 128).

Cercopithecus callitrichus and *sabæus*, eggs, d'Athias (1915, p. 68).

Lepus cuniculus, *Mus musculus* and *decumanus*, *Cavia cubaya*, *Felis domestica*, *Homo sapiens*, L. and R. Zoja (1891, p. 256).

PLANTS.

Myxomycetes: ———.

Schizophytes: ———.

Algae:

Cystoseira barbata, apical cell, Nicolosi-Roncetti (1912b, p. 144).

Fucus serratus, apical cell, Le Touze (1912, plate 9).

Gastroclonium reflexum, *Gigartina teedii*, *Lemanea torulosa*, carpospores and tetraspores, Nicolosi-Roncetti (1912a, p. 60 (?)).

Vaucheria, filaments, Rudolph (1912, plate 18).

Coccomyxa solonina, mitochondria absent, the Moreaux (1916, p. 242).

Fungi:

Achlya, hyphae, Rudolph (1912, plate 18).

Albugo bliti and *candida*, hyphae and conidia, Lewitsky (1913, pl. 21).

Aleuria cerea, asci, Guilliermond (1913a, p. 618).

Botrytis cinerea, Guilliermond (1913g, p. 1781).

Colco-sporium senecionis, Mme. Moreau (1914, p. 421).

Endomyces magnusi, fibuliger, and *albicans*, Guilliermond (1913g, p. 1781).

Entyloma ranunculi, Moreau (1914b, p. 538).

Penicillium glaucum, Guilliermond (1914b, fig. 10).

Peziza leucomelas, asci, Guilliermond (1913c, p. 65).

Peziza catinus, asci, Guilliermond (1913a, p. 618).

Fungi—continued.

Phragmidium subcorticium, telautospore, Mme. Moreau (1914, p. 421).

Psalliota campestris, hyphae, basidia, and spores, Beauverie (1914a, p. 800).

Puccinia malvacearum, hyphae, Beauverie (1914b, p. 359).

Pustularia vesiculosa, asci, Guilliermond (1913a, p. 618; 1913b, p. 646).

Rhizopus nigricans, young spores and filaments, Moreau (1915b, p. 143).

Saccharomyces cerevisiae and *ludwigi*, cells, Guilliermond (1913g, p. 1781).

Sporodinia grandis, zygospores, Moreau (1914a, p. 347).

Bryophytes:

Anthoceros hushoti, several parts, Scherrer (1913, pl. 20).

Bryum ———, Sapehin (1915, pls. 25, 26).

Funaria hygrometrica, leaves, Sapehin (1915, pls. 22, 23, 25, 26).

Polytrichum piliferum, Sapehin (1915, pls. 22, 25).

Pteridophytes:

Aspidium felix-mas, young leaf, Pensa (1911, fig. 3; 1912, pls. 27, 28).

Azolla, *Mirande* (1916, p. 369).

PLANTS—Continued.

Pteridophytes—continued.

- Lycopodium inundatum*, Sapehin (1915, pl. 12).
Selaginella vulgare, leaf, Pensa (1911, figs. 4, 5; 1912, pl. 27).
Selaginella martensi, leaf, Löwschin (1914, p. 268).
- Spermatophytes:
- Allium cepa* and porrum, root-tip, Duesberg and Hoven (1910, p. 99).
Ampelopsis vietchi, leaf, Guilliermond (1913f, p. 1000; 1914a, p. 566).
Amaryllis, ovary, Guilliermond (1912c, p. 888).
Anagallis arvensis, petal, Moreau (1914c, p. 502).
Asparagus officinalis, several portions, Lewitsky (1910, pl. 17), Rudolph (1912, p. 18), Sapehin (1915, pl. 10).
Asparagus sprengeri, buds, Guilliermond (1914a, p. 566).
Aucuba japonica, buds, Pensa (1911, fig. 1).
Begonia, *Camelia*, *Canna*, buds, ovaries, and flower, Guilliermond (1912c, p. 888; 1914a, p. 566).
Cannabis sativa, cotyledon, Löwschin (1914, p. 268).
Cerasus Laurocerasus, bud, Guilliermond (1912a, p. 365).
Chenopodium amaranticolor, leaf, Guilliermond (1914b, figs. 10, 11).
Corylus, leaf, Löwschin (1914, p. 268).
Curcubita pepo, hair, Maximow (1913, figs. 1-9).
Dahlia, flower, Guilliermond (1914a, p. 566).
Daucus carota, root, Guilliermond (1912b, p. 411; 1914a, p. 566).
Echeveria glauca, epidermal and hypodermal cells, Rudolph (1912, p. 619).
Elodea canadensis, several parts, Lewitsky (1911, pl. 28).
Erythrina, pollen mother-cells, Guilliermond (1912c, p. 890).
Euonymus japonicus, buds, Guilliermond (1912a, p. 365).
Euphorbia myrsinitis, meristem of stem, Sapehin (1915, pl. ii).
Ficaria ranunculoides, root, stem, leaf, Guilliermond (1913d, p. 439; 1914a, p. 566).
Ficus elastica, leaf, Guilliermond (1914a, p. 566).
Fritillaria imperialis, ovary and pollen, Orman (1912, pls. 1, 3, 4).
Gladiolus, ovary, Pensa (1910, p. 326).
Helleborus fetidus, tapetum, Nicolosi-Roncati (1910, p. 109).
Hordeum, Guilliermond (1912d, p. 86).
Hyacinthus orientalis, root, von Smirnow (1906, p. 146).
Hydrangea vestita, winter buds, Pensa (1912, pls. 26, 28).
Ilex aquifolium, leaf, Guilliermond (1914a, p. 566).
Iris germanica, parts of flower, Guilliermond (1915, p. 241).

Spermatophytes—continued.

- Juglans regia*, secreting hair-cells and young leaf, Guilliermond (1914a, p. 566).
Lilium candidum, ovule, Pensa (1911, fig. 2; 1912, pl. 25).
Lilium croceum, Orman (1912, pl. 2).
Lilium martagon, ovary, Pensa (1910, p. 326).
Lilium rubrum, ovary, Guilliermond (1912c, p. 888).
Lupinus albus, Pensa (1912, pl. 27).
Lychnis dioica, hairs on stem, Moreau (1914c, p. 502).
Lycopersicum pyriforme, fruit, Guilliermond (1914a, p. 566).
Mormordica elaterium, hair-cell, Zimmermann (1893, p. 215, fig. 2).
Nereum oleander, leaf, Guilliermond (1914a, p. 566).
Nymphaea alba, tapetum, Meves (1904, p. 284).
Oenothera biennis, several parts, Sapehin (1915, pls. 11, 12).
Papaver rhoeas, ovary, Pensa (1910, p. 326).
Phajus grandifolius, root, Guilliermond (1914a, fig. 6).
Phaseolus vulgaris, root tip, Duesberg and Hoven (1910, p. 97).
Philodendron grandifolium, leaves, Guilliermond (1914a, p. 566).
Pinguicula hirtiflora, gland-cells, Nicolosi-Roncati (1912c, p. 184).
Pisum sativum, Duesberg and Hoven (1910, figs. 1-5).
Polygonum aviculare, stipule, Moreau (1914c, p. 502).
Populus tremula, leaf, Löwschin (1914, p. 268).
Quercus pedunculata, leaf, Löwschin (1914, p. 268).
Ricinus, *Ricinus gibsoni*, various parts, Guilliermond (1914a, p. 566).
Rosa, leaf bud, Pensa (1913a, p. 81).
Rosa thea, ovary, Pensa (1910, p. 326).
Sedum telephium and reflexum, epidermis and hypodermal cells, Rudolph (1912, p. 619).
Solanum tuberosum, ovary, Pensa (1910, p. 326).
Taxus baccata, bud, Pensa (1911, p. 528; 1912, pl. 25).
Tilia parvifolia, leaf, Löwschin (1914, p. 268).
Triticum, Guilliermond (1912d, p. 86).
Tradescantia discolor, root, Guilliermond (1914a, p. 566).
Tradescantia virginica, Duesberg and Hoven (1910, p. 99).
Trianea bogotensis, hairs of root, Sapehin (1915, pl. 11).
Tropaeolum lobbeum, portions, Guilliermond (1914a, p. 566).
Tulipa gessneriana and *sylvestris*, ovule, etc. Pensa (1912, pls. 25, 28; 1911, figs. 6, 7).
Tulipa suaveolens, Guilliermond (1917, p. 233).
Vanilla planifolia, stem and leaves, Guilliermond (1914a, p. 566).
Vicia faba, root meristem, Zimmermann (1893, p. 215).
Yucca filamentosa, ovary, Pensa (1910, fig. 4).
Zea mais, root, Guilliermond (1912d, p. 86).

IV. TECHNIQUE.

EXAMINATION OF LIVING CELLS UNSTAINED.

There is no longer any question of the actual existence of mitochondria in living cells. They may be studied in cells teased out in serum or physiological salt solution, with direct illumination. It is possible to see them more easily in some cells than in others on account of some variation in their refractive index or that of the cytoplasm in which they are embedded. A remarkable phenomenon is frequently seen in the pancreas and other tissues; the mitochondria, instead of being visible with difficulty, flash out sharply and can be seen with the greatest clearness, even though they are quite unstained. This may be due to a decrease in saturation, because we are told that in substances like them outside the organism, a decrease in saturation is accompanied by an increase in refractive index. Guilliermond (1912a, p. 409) thinks that the high refractive index which the mitochondria sometimes possess results from the peculiar combination of lipoid with the albuminous substratum of which they consist, which in his opinion may be disposed on the surface, in the form of a fatty membrane.

Mitochondria may also be examined with the ultra-microscope (as well as by ordinary dark-field illumination) in the manner suggested by Regaud. No systematic attempts have been made to study them with light of different wavelengths. One of the best ways to study mitochondria in living cells is by means of the tissue-culture method as advocated by the Lewises (1915, p. 339).

VITAL STAINING.

It has been claimed by Tschaschin (1912, p. 304) that the mitochondria may be stained vitally with isamine blue (=pyrrol blue); but Scott (1915, p. 835) demonstrated that this was not the case by bringing to light the mitochondria in these blue-stained cells with janus green. Nevertheless Levi (1916a, p. 107), still more recently, has come to the conclusion that the mitochondria in the cells of tissue cultures may be stained with pyrrol blue. This confusion is due, in the first place, to mistakes in the identification of mitochondria, and, in the second place, to a lack of any general consensus of opinion as to just what constitutes a staining of mitochondria. Some investigators would speak of mitochondria as being specifically stained when others would be loath to do so; some are content with a mere tingeing of mitochondria, which can with difficulty be seen and which others would overlook. My own experience is that almost any coloring matter will stain the mitochondria if it is used in sufficient concentration, and I am not at all sure that the controversy is a profitable one.

It is quite possible that in some cases the mitochondria are stained while in others they are not. There is no *a priori* reason why the mitochondria should not take up these vital dyes. In fact, such action would be in complete accordance with Regaud's ectosome theory (p. 118).

SUPRAVITAL STAINING.

The most satisfactory dyes for this purpose are janus green B, janus blue, janus black I, and diethylsafranin (Cowdry, 1916*a*, p. 430). Their action is similar and is described in detail on page 92. They may be applied by immersion or injection.

IMMERSION.

The best results are obtained with blood (Cowdry, 1914*b*, p. 267) as follows:

Janus green B should be employed in a concentration of about 1:10,000 in 0.85 per cent sodium-chloride solution. A drop should be placed on a series of six or more slides. A small amount of freshly drawn blood is then added to the dye and a cover-glass is immediately dropped on it. No attempt should be made to mix the blood with the stain before covering.

The preparations should now be examined. Almost immediately one of them will begin to show mitochondria, first in the lymphocytes and later in the granular leucocytes. Soon the mitochondria will be stained in all of them. Under favorable conditions they will last for several hours. Evaporation may be reduced by putting a ring of vaseline around the edges of the cover-glass.

INJECTION.

This method is most satisfactory with the pancreas (Bensley, 1911, p. 304) and salivary glands.

The animal is killed and janus green B is injected into the left ventricle or aorta in a concentration of 1:10,000 of salt solution by gravity pressure. In order to obtain a good penetration the return flow through the inferior or superior vena cava, as the case may be, should be momentarily cut off by artery clamps. After about 10 minutes' perfusion, small portions of the gland may be removed and examined for mitochondria. When the desired intensity of staining has been reached, the entire gland should be placed in salt solution pending examination.

FIXATION AND STAINING.

(1) Altmann's (1890, p. 27) anilin-fuchsin-picric acid method (slightly modified):

Fixation:

- (1) Fix small fragments of not more than 2 c. mm. in equal parts of 5 per cent potassium bichromate and 2 per cent osmic acid for 24 hours.
- (2) Wash in water, 1 hour.
- (3) Dehydrate in 50 per cent, 70 per cent, 95 per cent, and absolute alcohol 24 hours each.
- (4) Half absolute alcohol and xylol, 3 hours.
- (5) Xylol, 3 hours.
- (6) 60° C. paraffin, 3 hours. Embed. Cut sections 4 μ , and fix to slides by albumen-water method.

Staining:

- (1) Pass down through toluol, absolute alcohol, 95 per cent, 70 per cent, and 50 per cent alcohol, about 30 seconds each, to aq. dest. in staining jars.
- (2) Stain for 6 minutes in Altmann's anilin fuchsin (anilin water 100 c.c., acid fuchsin 20 gm.). The stain may be poured onto the slide and the whole gently heated over a spirit lamp.
- (3) Blot and differentiate by carefully flooding the section with a mixture of 1 part of sat. alc. solution of picric acid and 2 parts of aq. dest., added with a pipette. During this operation the color can be best seen against a white background.
- (4) Rinse rapidly in 95 per cent alcohol. Pass through several changes of absolute into xylol and mount in balsam.

In this way the mitochondria are stained a beautiful crimson color against a bright yellow background. It is the oldest and in many respects the best of mitochondrial methods; but it has two disadvantages—the fixative penetrates badly and the colors fade rapidly. Accordingly, neutral balsam or damar should be used, the specimens should not be exposed to direct sunlight or to heat, and they should be kept in a dry place.

This method has been greatly modified by Galeotti (1895, p. 466), Schridde (1905, p. 696), Bensley (1911, p. 308), Freifeld,¹ Kull (1913, p. 153), Schirokogoroff (1913, p. 523), Cowdry (1916*b*, p. 30), Duesberg (1917, p. 469), and others.

Bensley proceeds as follows:

Fixation:

- (1) Fix in acetic, osmic, bichromate mixture (2.5 per cent potassium bichromate 8 c.c., 2 per cent osmic acid 2 c.c., glacial acetic acid 1 drop) for 24 hours.
- (2) Wash, dehydrate, clear, and embed (p. 59), except that bergamot oil is substituted for xylol.

Staining:

- (1) Pass down to water.
- (2) Dip in 1 per cent potassium permanganate about 1 minute.
- (3) Rinse in 5 per cent oxalic acid same time and wash in water.
- (4) Stain with anilin fuchsin as indicated (p. 59).
- (5) Differentiate in a 1 per cent aqueous solution of methyl green.
- (6) Rinse rapidly in 95 per cent alcohol. Pass through several changes of absolute into xylol and mount in balsam.

The use of permanganate and oxalic acid corrects excessive mordanting with the osmic and bichromate. It may sometimes be dispensed with. The methyl green, which was first used in this way by Galeotti, is a much finer contrast stain than the pieric acid and is also more permanent. The precautions already mentioned against fading should be observed.

I make use of the following modification:

Fixation:

- (1) Fix in Regaud's mixture (4 parts of 3 per cent potassium bichromate and 1 part of commercial formalin). The commercial formalin may profitably be neutralized by saturation with magnesium carbonate. The mixture may be applied by immersion or injection, the latter being recommended for large objects. It should be changed every day for 4 days and be kept in an ice-box (though this is not essential). Mordant for 8 days in 3 per cent potassium bichromate, changing every second day.
- (2) Wash in running water over night.
- (3) Dehydrate, clear, and embed as indicated (p. 59).

Staining:

- (1) Pass slides to water as indicated (p. 59).
- (2) 1 per cent potassium permanganate 30 seconds, but time must be determined experimentally.
- (3) 5 per cent oxalic acid 30 seconds. NOTE.—Steps (2) and (3) may usually be dispensed with.
- (4) Rinse in several changes of distilled water about 1 minute. Incomplete washing prevents staining with fuchsin.
- (5) Stain in Altmann's anilin fuchsin made up as follows: Make a saturated solution of anilin oil in distilled water by shaking the two together. Filter and add 10 gm. of acid fuchsin (Duesberg) to 100 c.c. of the filtrate. The stain should be ready to use in about 24 hours. It goes bad in about a month. To stain, dry the slide with a towel, except the small area to which the sections are attached; cover the sections on the slide with the stain and heat over a spirit lamp until fumes, smelling strongly of anilin oil, come off; allow to cool; let the stain remain on the sections about 6 minutes; return the stain to the bottle.
- (6) Dry off most of the stain with a towel and rinse in distilled water, so that the only remaining stain is in the sections. If a large amount of the stain is left it will form a troublesome precipitate with methyl green; on the other hand, if too much stain is removed the coloration of the mitochondria will be faint.
- (7) Allow a little 1 per cent methyl green, added with a pipette, to flow over the sections, holding the slide over a piece of white paper, so that the colors may be seen. Apply the methyl green for about 5 seconds at first and modify as required. This is the crucial point of the method.
- (8) Drain off excess of stain, plunge into 95 per cent alcohol for a second or two. Then rinse in absolute alcohol, clear in toluol, and mount in balsam.

Difficulties.—(1) The methyl green may remove all the fuchsin, even when applied only for a short time. This is due to incomplete mordanting of the mito-

¹For which see Naegeli (1912, p. 30).

chondria by the chrome salts in the fixative. It may be avoided by omitting steps (2) and (3), or by treating the sections with 2 per cent potassium bichromate for a few seconds just before staining (as advised by Bensley). The action of the permanganate and oxalic is to remove the bichromate. (2) The fuchsin may stain so intensely that the methyl green removes it imperfectly or not at all. This, on the contrary, is due to too much mordanting. It may be corrected by prolonging steps (2) and (3). (3) Sometimes, after obtaining a good differentiation, the methyl green is washed out before the slide is placed in toluol, in which event omit the 95 per cent and pass to absolute direct.

This fixative is a good penetrator, in which respect it is much superior to Altmann's fluid or Bensley's mixture; but it makes the tissues very brittle and difficult to cut. The staining is satisfactory and uniform.

(2) Benda's (1901, p. 155) crystal violet alizarin method:

Fixation:

- (1) Flemming's fluid, 8 days.
- (2) Wash 1 hour, half pyrolineous and 1 per cent chromic acid, 24 hours.
- (3) 2 per cent potassium bichromate, 24 hours.
- (4) Wash in running water 24 hours, dehydrate, and embed in paraffin. Cut sections $\epsilon\mu$.

Staining:

- (1) 4 per cent iron alum, 24 hours.
- (2) Rinse in water and bring into an amber-colored solution of sodium sulphalizarinate, made by adding a saturated alcoholic solution to water, 24 hours.
- (3) Blot with filter-paper and stain in equal parts of crystal violet solution and water. (N. B.—The crystal violet solution made of conc. sol. crystal violet in 70 per cent alcohol 1 volume, alcohol 1 volume, and anilin water 2 volumes.
- (4) The solution is warmed until the vapor arises and then allowed to cool for 5 minutes.
- (5) Blot, then 30 per cent acetic acid 1 minute.
- (6) Blot, plunge in absolute alcohol until but little more stain comes off, clear in xylol, and mount in balsam.

A useful modification is given in Meves and Duesberg (1908, p. 573). Successful Benda preparations are excellent. The mitochondria are stained a deep violet color against a rose background. They are also much more permanent than Altmann preparations. Unfortunately the method is long, tedious, and difficult.

(3) Regaud (1910, p. 296) has used the iron-hematoxylin method of Heidenhain after a large variety of fixatives, the best of which is his formalin and bichromate mixture.

Fixation:

- (1) Fix in 3 per cent potassium bichromate 80 volumes, commercial formalin 20 volumes, for 4 days, changing every day.
- (2) Mordant in 3 per cent bichromate for 7 days, changing every second day.
- (3) Wash in running water 24 hours, dehydrate, clear, and section as indicated (p. 59).

Staining:

- (1) Pass down to water as indicated (p. 59).
- (2) Mordant in 5 per cent iron alum at 35° C. for 24 hours. Rinse in aq. dest.
- (3) Stain for 24 hours in hematoxylin made up as follows: Dissolve 1 gm. pure crystals of hematoxylin in 10 c.c. of absolute alcohol and add 10 c.c. of glycerin and 80 c.c. of distilled water.
- (4) Differentiate in 5 per cent iron alum under microscope.

NOTE.—The crucial point in the technique is passing from the mordant to hematoxylin. The slides must be rinsed in *distilled* water, otherwise the iron alum will form a dense black precipitate in the stain. On the other hand, if they are rinsed too much, all the iron alum mordant will be removed. It is necessary to strike the happy mean in which a darkening of the hematoxylin alone occurs. It is always difficult to get good hematoxylin, and I find it best to keep on hand a ripe alcoholic solution.

This is the most permanent as well as the simplest of all mitochondrial stains. It may be used in the damp climates of most of our marine biological laboratories,

where the Altmann method and its modifications are useless. Unfortunately it rarely gives good results with embryonic tissues; for these the older osmic acid-containing fixatives are best adapted. It is often possible to make use of material fixed in the usual way with formalin by starting out with step (2). Moreover, the preparations can be counterstained in a variety of ways (Cowdry, 1916a, p. 441).

(4) Dubreuil's (1913, p. 74) iron-hematoxylin method for blood-cells:

Fixation:

Take up the fluid to be examined in a pipette containing several times its volume of 0.5 to 1 per cent solution of osmic acid. Transfer to a centrifuge tube. A good fixation is obtained in about an hour. Then add distilled water, centrifuge, and decant. The blood-cells remaining are shaken up with absolute alcohol and then passed into a weak solution of celloidin. A drop is allowed to spread on a slide, which, before complete desiccation, is plunged into 80 per cent alcohol.

Staining:

The mitochondria in the cells are then stained with iron hematoxylin (p. 61).

(5) Bensley's copper chrome hematoxylin method:

Fixation:

- (1) Fix in either Altmann's osmic bichromate mixture (p. 59) or in Bensley's acetic-osmic-bichromate fluid (p. 60) for 12 to 24 hours.
- (2) Wash, dehydrate, clear, embed, and section (p. 59).

Staining:

- (1) Pass down to water (p. 59).
- (2) Sat. aq. copper acetate, 5 minutes.
- (3) Wash in several changes distilled water, 1 minute.
- (4) 0.5 per cent hematoxylin, 1 minute. (If the copper acetate has not been sufficiently washed out, a black precipitate forms in the hematoxylin. The hematoxylin should be well ripened. It may be obtained by dilution down from a 10 per cent alcoholic stock solution.)
- (5) Rinse in aq. dest.
- (6) 5 per cent neutral potassium chromate, 1 minute. (The sections should turn a dark blue-black color. If they are only a light-blue shade, rinse in aq. dest., place again in the copper acetate, and carry through as just described several times until no increase in color results.)
- (7) Wash in aq. dest. and return for a few seconds to the copper acetate in order to convert all the dye into the copper lake.
- (8) Wash in aq. dest.
- (9) Differentiate under the microscope in Weigert's borax-ferriyanide mixture diluted with 2 volumes of aq. dest.
- (10) Wash 6 to 8 hours in tap water.
- (11) Dehydrate, clear, and mount in balsam.

(6) Bensley's (1911, p. 308) neutral safranin method:

Fixation:

- (1) Fix in chrome sublimate (2.5 per cent potassium bichromate 100 c.c., mercuric chloride 5 gm.) for 24 hours.
- (2) Wash, dehydrate, clear, embed, and section as indicated on page 59.

Staining:

- (1) Preparation of stain: Add slowly sat. aq. sol. of the color acid, acid violet, to sat. aq. sol. of the color-base, safranin O, contained in a flask until a precipitate no longer forms. The point of neutralization may be roughly determined by dropping a little of the mixture on filter-paper from time to time until the outside red ring of safranin disappears and the whole blot takes on a neutral color. Filter. The filtrate should be as nearly as possible colorless. Dry the precipitate on filter-paper for 12 hours, collect it, and make a saturated solution of it in absolute alcohol.
- (2) Pass sections down through two changes of toluol and absolute alcohol in order to remove all traces of paraffin or toluol, which might interfere with the staining. Then through 95 per cent, 70 per cent, and 50 per cent to aq. dest. (Chrome and osmium fixed material must be bleached in permanganate and oxalic acid (vide p. 60), and sublimate-fixed tissues must be treated with Lugol's iodine solution for about 10 seconds and washed in aq. dest.)
- (3) Dilute the alcoholic stock solution of the dye with an equal volume of aq. dest. and stain for from 5 minutes to 2 hours.
- (4) Blot quickly with several layers of filter paper.
- (5) Plunge into pure acetone and pass immediately to toluol without waiting to drain.
- (6) Examine under the oil immersion and if necessary differentiate in oil of cloves. If this is not sufficient, the slide, after rinsing in absolute alcohol, may be instantaneously flooded with 95 per cent alcohol, and then passed back through absolute alcohol to toluol.
- (7) Wash in two changes of toluol and mount in balsam.

Working on the same principle, a number of stains can be made up for mitochondria (Cowdry, 1913*a*, p. 485). Note also Bensley's neutral gentian method.

(7) Bensley's (1916, p. 47) brazilin-wasserblau method:

Fixation:

- (1) Fix in Zenker's fluid, plus less acetic acid, 10 per cent formalin, 24 hours.
- (2) Wash, dehydrate, clear, embed, and section (p. 59).

Staining:

- (1) Pass down to water (p. 59).
- (2) Iodize with Lugol's solution, 30 seconds.
- (3) Stain in following solution several hours: Phosphotungstic acid, 1 gm.; aq. dest., 100 c.c.; brazilin, 0.05 gm. The brazilin is first dissolved in a small quantity of distilled water by the aid of heat and added to the phosphotungstic-acid solution. Ripening may be accelerated by the addition of 0.4 c.c. of hydrogen peroxide, or of a few drops of a solution of soluble molybdic acid. The solution deteriorates with age and should not be used after 3 days.
- (4) Rinse in aq. dest. and place for 1 to 5 minutes in phosphotungstic acid, 1 gm.; wasserblau, 0.2 gm.; aq. dest., 100 c.c.
- (5) Wash rapidly in water, dehydrate in absolute alcohol, clear in toluol, and mount in balsam.

(8) Meves's (1905, p. 102) new Victoria green method: This method is intended for red blood-cells which are simply stained in the fresh condition by the addition of a 4 per cent iodine-acid solution to which a small quantity of new Victoria green (malachite green) has been added.

(9) The methods of silver reduction employed by many Italian investigators are essentially modifications of the original method of Golgi. They undoubtedly reveal mitochondria in most cases, but one would hesitate to attribute any high degree of specificity to them. For details see Veratti (1909, p. 34), Pensa (1910, p. 326), Rina Monti (1915, p. 21), and Cajal (1915, p. 3).

For still other mitochondrial methods see Sjovall (1906, p. 563), Rubaschkin (1910, p. 407), Koltzoff (1906, p. 384), Kingsbury (1911, p. 317), Schultze (1911*a*, p. 465), Maximow (1916*a*, p. 462), and others.

In unskilled hands the experimental error in some of these methods of technique is sometimes very great, but it is a mistake to regard the methods as difficult.

In the examination of living cells it is of course essential that the medium should be as nearly as possible isotonic. The presence or absence of Brownian movement is a valuable criterion of the condition of the cells. Where it is very marked the material should be discarded, because it indicates that an unusually large amount of water has entered into the cells. Each particle, instead of being held in place by the balanced action of many bombarding molecules, is subject to the action of only a few, now on one side and now on the other, causing it to jump from place to place. The smaller the particle the less chance there is of molecules on the opposite side compensating. Large granules, on the other hand, offer a greater surface, are more likely to be bombarded from all sides, and are thereby held in place and do not show so much tendency toward exhibiting Brownian movement.

One of the most common and annoying sources of error is mechanical manipulation of the fresh tissue before fixation. A mere crushing of the tissue with the forceps will bring about the most astonishing and perplexing modifications in the mitochondria. Allowing a surface film of the tissue to dry in air, as it stands on the autopsy table, is another common blunder which changes the whole appearance of the mitochondria. Osmotic changes in the tissues before fixation must also be

carefully guarded against. Merely keeping the tissue in salt solution will often cause the mitochondria to assume the most bizarre and perplexing forms. The tissues should be quite fresh, particularly the pancreas, in which autolysis takes place very rapidly. But the need for fresh tissue has been so much exaggerated that it has discouraged the study of mitochondria without reason. In the nervous system, for example, where autolysis is slow, the mitochondria may be advantageously studied 6 or even 12 hours after death. Material awaiting fixation should be kept in a cool place and precautions taken against evaporation.

Poor penetration of the fixative is also well known to bring about definite changes in the mitochondria. The mitochondria in the deeper parts of the tissue become spherical and swell up. Indeed, when a fixative like the acetic-osmic-bichromate mixture of Bensley is applied simply by immersion the action of each of the ingredients may be seen. On the very surface the usual filamentous form of mitochondria is maintained through the combined action of osmic acid, bichromate, and acetic; a little deeper in, where the osmic acid has not penetrated, the bichromate and acetic alone acting, the mitochondria are swollen up and spherical; while in the central parts of the tissue the mitochondria have been completely dissolved through the unmodified action of the acetic acid. Simultaneous action of the ingredients is satisfactory, but successive action is not. One comes across varying degrees of artifact as one proceeds inward. Naturally, small pieces of tissues alone can be used. They should be of not more than 4 cubic millimeters, and it is better to have even smaller when the tissues are hard and firm and resist the penetration of the fixative. It is unfortunate that investigators do not avail themselves more generally of the method of applying the fixative by injection through the blood-vessels. Naturally this can not be done with osmic acid on account of the expense, but the mixtures of formalin and bichromate advocated by Regaud will give excellent and uniform fixation in very tough and fibrous tissue when applied by vascular injection. It is always necessary to wash out the blood with salt solution before applying the fixative.

That the concentration of the fixative is also of great importance has been shown by N. H. Cowdry (1917, p. 200), who finds that very dilute solutions of formalin cause a swelling of mitochondria and concentrated ones a shrinkage. It is likely that most mitochondrial methods bring about a slight shrinkage of the mitochondria.

Now, the fixative in the right concentration having come in contact with the mitochondria, it becomes necessary to inquire whether it preserves them in their true form without distortion. This is a difficult question to answer. In the majority of cases with care and good fixation the original form of the mitochondria is preserved. This was first shown with striking clearness by the Lewises (1915, p. 347), who studied the living cells in tissue cultures and then actually watched the process of fixation in them. When fixatives do alter mitochondria, they alter them in a definite way. Filamentous mitochondria break up into granules and spherules. Filamentous mitochondria are never formed by the fixative from granules.

Experience teaches that a great polymorphism of mitochondria in a preparation of cells of the same type, acinus cells of the pancreas for instance, is usually the result of experimental error before or during fixation. Such preparations should be discarded unless the polymorphism occurs constantly with different fixatives or can be seen in the living condition.

Another error which frequently creeps in is a variation in mordanting which manifests itself in the subsequent staining reactions, in the interpretation of which the mordanting must always be borne in mind. But perhaps the most important variable is differentiation. Preparations assume an entirely different aspect with the degree of differentiation. This is particularly true where the differentiator itself is colored, as in the case of methyl green.

Let us bear in mind that not one of these methods is specific for mitochondria. Janus green is the most specific, but it will stain other structures (p. 59) under certain conditions. The iron-hematoxylin method of staining is certainly the least specific of all, because it even colors chromatin in the same way as mitochondria. Neither is the Altmann method specific. There are fewer objections to its fuchsin-methyl green modification and to the Benda method, though neither of them can be completely trusted.

Finally, a word as to the relative value of observations on fixed and fresh material. It is the fashion now to insinuate that the older methods of fixation and staining are more or less useless. This should be deplored. It may be said that the value of fixation consists in the rapidity of its action. The cell is killed instantaneously and a condition resembling more or less closely that of the living cell at a definite moment in the course of its normal functional cycle is preserved. On the other hand, in the observation of living tissue, the much-prolonged time factor constitutes a serious and unfortunately but little recognized source of error. A half hour spent in studying mammalian tissues teased out in so-called isotonic media gives abundant opportunity for experimental error to creep in. Nevertheless, the study of fresh tissue contributes invaluable information as to the process which is taking place, even though the process can not be regarded as entirely normal. The other great and unique advantage which the observation of living tissue presents is that it permits of the experimental modification of the various vital processes going on in the cells. To sum up, the methods of fixation of mitochondria will never be replaced by the observation of living tissues, but they will be greatly supplemented, extended, and aided by it. The two must go hand in hand.

The essential thing about mitochondrial technique is the necessity of experimentation. There is nothing really difficult about it, but it is too much to expect to get good results after the first or second trial. For this reason attempts to shift the responsibility onto the shoulders of untrained technicians almost invariably result in mutual dissatisfaction.

V. MORPHOLOGY.

MORPHOLOGY IN ORGANISMS.

We rarely meet with forms which possess mitochondria of peculiar or distinctive morphology. In multicellular animals and plants, high up in the scale, in which there is considerable division of labor among their constituent parts, all forms of mitochondria are represented, though some may predominate. In unicellular organisms, on the other hand, the mitochondria are sometimes granular, sometimes filamentous, sometimes large and sometimes tiny, depending upon conditions which are wholly obscure. Frequently we meet with all forms within the compass of a single cell. Obviously they can never be used in a taxonomic way, like the chromosomes, to distinguish between nearly related species. This is equally true for animals and for plants.

MORPHOLOGY IN TISSUES.

The several tissues of the higher plants and animals possess mitochondria of characteristic form; that is to say, in some of them filaments predominate, in others granules, and so on, but in similar tissues of different animals they are much the same. For instance, I find it difficult, even in the different classes of vertebrates, to distinguish the spinal ganglion cells from each other on the basis of their mitochondrial content alone. The mitochondria in the liver, pancreas, lung, prostate, and other organs are characterized by the predominance of some definite form, granule, rod, or filament in all nearly related animals. This general constancy of mitochondria, where the function is similar, is, I think, of considerable importance, because it must surely indicate that their morphology is a fundamental property ingrained in the very organization of the cell in phylogeny and that it is not always a passing trivial affair which varies from moment to moment.

Mitochondria are for the most part *filamentous* in certain nerve-cells (plate 1, fig. 4), in gland-cells (plate 1, fig. 9), and others, as well as in most of the tissues of the developing embryos of all forms. Indeed, filamentous mitochondria are among the most common met with anywhere. The average length of the filaments varies in different tissues: they are perhaps longest in secreting cells, like the pancreas, where they may attain a length of 10 to 12 μ . Their diameter also varies (0.05 to 0.2 μ) in different localities, but in individual cells of the same tissue it is astonishingly uniform. Within a single cell the length is variable and the breadth uniform. They may be straight, curved, or even twisted, depending upon their surroundings. They do not begin to taper toward their extremities, which end abruptly and evenly. Sometimes their ends are somewhat swollen.

Filaments are never produced from granules through the action of the fixative. Some think that they are formed, in the living tissue, by linear coalescence of individual granules; others, that they arise from single granules through growth by accretion. Certain it is that in those cells where filaments are abundant all transitions may be seen between them and granules, while filaments are often totally absent in cells with granular mitochondria.

There is a tendency among investigators to believe that filamentous mitochondria are the direct result of streaming movements in the cytoplasm. Certain cases may be cited in support of this theory, like the outgrowing nerve-fiber, for instance, in which the mitochondria are always filamentous. In gland-cells also they are usually filamentous and stretch from the basement membrane toward the lumen in the direction of the passage of materials from the blood-stream into the gland-duets. Again, in dividing cells, the mitochondria often stretch out in the direction of separation. But we have other cases, which demand explanation, in which the mitochondria are rod-like or even granular, in spite of the streaming movements; and still other cells where the mitochondria are filamentous, though the cytoplasm is to all intents and purposes stationary, or at any rate as motionless as it ever is. Thus, the mitochondria are granular and rod-like, not filamentous, in living human polymorphonuclear leucocytes during amœboid movement and in the streaming protoplasm of certain plants. On the contrary, in sharp contrast are cartilage-cells and bone-cells, where, so far as we know, the cytoplasm is relatively quiescent, yet the mitochondria are filamentous. They remain thread-like in the nerve-fiber throughout the whole life of the animal, even though there is no longer a pushing-out of substance. Nicholson (1916, p. 332) has found that they are constantly filamentous in the bodies of some nerve-cells, granular in others; and that where they are filamentous they are more so in the peripheral cytoplasm (where they are disposed parallel to the cell-wall) than in the deeper cytoplasm about the nucleus. If their shape is conditioned by protoplasmic streaming, our conclusion must be that some types of nerve-cells constantly exhibit it, that others never do, and further that the little vortices are most powerful in the peripheral cytoplasm, where the mitochondria constitute a clue to their direction. And this is not the end of the interesting deductions which would follow. While some workers are inclined to pin their faith in the real existence of such currents in nerve-cells, Kite's¹ microscopic dissections with very fine glass needles have brought to light the fact that their cytoplasm is very viscid and has the physical consistency of a gel. Furthermore, Key's² observation that it is difficult to alter the distribution of cytoplasmic materials in nerve-cells by centrifuging indicates the improbability of any considerable protoplasmic streaming.

Rubaschkin's (1910, p. 428) idea that filamentous mitochondria are characteristic of specialized cells and granular ones of embryonic, undifferentiated cells has been negated again and again; and Dubreuil's (1913, p. 137) view that filamentous ones are indicative of rest and granular ones of rapid multiplication by division is not borne out by recent work. According to the descriptions of Moreau (1914*b*, p. 538) they are granular in the spores of the fungi in which the cell activities are at a very low ebb, and N. H. Cowdry finds that they are filamentous in the more or less inactive cells of the radicle of the dried seed pea. We have here two instances of mitochondria in quiescent protoplasm; in one they are granular and in the other filamentous. Numerous other instances might be cited to show that filamentous mitochondria are not indicative of rest.

¹ Public lecture at the Marine Biological Laboratory, Woods Hole.

² Personal communication.

Filamentous mitochondria with bleb-like swellings are to be found in some secreting cells, but not in all. The blebs are supposed to be the precursors of secretion. In plant cells, starch, pigments, and other materials are unquestionably laid down in them, while in animal cells they are said to give rise to granules of zymogen. Many cells which form a definite secretion (plate 1, figs. 3 and 7) do not possess mitochondria armed with these swellings. I refer also to mucous cells. On the other hand, while filamentous mitochondria are exceedingly common, they never, to the best of my knowledge, have blebs in cells which do not form some distinct secretion. I have in mind particularly the nervous system, where mitochondria of this kind are unknown. The swellings are certainly not the result of technique; they represent a reaction on the part of mitochondria to some definite and specific demand or condition of the cell.

In other localities mitochondria are *rod-like*. They vary in length and in girth. They are long and very straight in the cells of the convoluted tubules of the kidneys, where they apparently constitute the well-known bâtonnets of Heidenhain. Here also they are of very large diameter. Smaller rod-like mitochondria are often to be seen in muscle-cells between the fibrils, in some nerve-cells, in fact in almost all tissues. Sometimes a swelling will develop at one end of a rod and in this way a *pear-shaped* mitochondrion will be formed. Occasionally they are formed through the action of the fixative upon mitochondria of the filamentous variety. The fixation may also modify their size to a certain extent; it may cause them to swell or to shrink, so that we must be on our guard.

Granular and dumb-bell-shaped mitochondria are of very common occurrence, though they are not so widely distributed as the filamentous and rod-like forms. They are quite spherical and resemble cocci rather than bacilli. It is sometimes difficult to distinguish between them and other cell inclusions on this account, because these other materials occur in the form of droplets also, rarely if ever as rods or filaments. Mitochondria of this variety are very abundant in some egg-cells (plate 1, fig. 6). The absence of very minute granular mitochondria merging into the invisible is of interest from the point of view of an origin *de novo* through condensation (page 98). The dumb-bells are usually interpreted as stages in the division of single granules, but it is unnecessary to say that they may equally well represent an approximation of two originally separate granules. Some fixatives will cause filamentous mitochondria to break up into granules, and I have even known janus green to bring about this change. Good fixatives which have poorly penetrated the deeper layers of pieces of tissue regularly cause this granular degeneration of mitochondria, so that the mitochondria become filamentous and gradually assume their normal condition as one approaches the periphery of the block.

Mitochondrial *networks* are of comparatively rare occurrence, though they are found normally in certain types of cells. They have been described in spermatogenesis, the Lewises (1915, p. 356) have seen them in tissue cultures, and I have occasionally seen them in the acinus cells of the pancreas of mice stained with janus green. They are of variable extent. They may consist of one or two meshes or of several. They are often, though not always, arranged about the nucleus.

Branching filaments are found in association with them and may constitute a stage in their formation. The strands of the network are of the same girth as the branching forms and of the individual filaments, except perhaps at the nodal points, where they may be slightly enlarged. It is still impossible to say whether the networks arise through a fusion of many mitochondria or by outgrowth of separate ones. They are difficult to preserve in fixed tissues by reason of the tendency of fixatives to cause fragmentation. Where they are found in fixed preparations we may be sure that they occurred in the living state. So far as we know at present, they are not associated with any definite function on the part of the cell.

Large spherical mitochondria are usually on the border-line between true mitochondria and lipid droplets. They represent a stage in the transformation and may be found in any tissue where the change is taking place. They may be seen to the best advantage in the nervous system, in the cells of the spinal ganglia, where all stages in the metamorphosis may be made out. When the mitochondria assume a diameter of about 3 or 4 μ they begin to become more and more resistant to acetic acid and clump together in the familiar lipid accumulations. The question is rather complicated, however, because large spherical mitochondria, of much the same appearance, may be produced by poor fixatives as well as by good fixatives which have penetrated badly. We must know the tissue. This warning is particularly applicable to *large spherical mitochondria with clear centers* and to *ring-shaped forms*, which should always be regarded with suspicion and interpreted with caution.

N. H. Cowdry (1917, p. 225) summarized his work on the morphology of mitochondria as follows:

"Their *morphology* is identical in plants and in animals; they assume no forms in the one which are not present in the other; they undergo similar variations in size and shape in different tissues and in different cells in both. If it were possible to view mitochondria dissociated from their environment, it would be impossible to decide whether they came from plant or animal tissues, provided they did not contain starch, pigment, or some other easily recognizable substance, to serve as a clue."

The little-known condition of chondriolysis is of interest. The mitochondria lose their definite form, whatever it may be, and give rise to a diffuse deposit of material staining with mitochondrial stains. It is of frequent occurrence in the nervous system in normal conditions and is conspicuous in other organs in pathological states (page 136). We should bear in mind the possibility that in all cells individual mitochondria go into solution and gradually fade away and reappear again, even though the change escapes our observation, except in special cases like that of the nervous system. Something similar has been described in the suprarenal by Mulon (1912, p. 36).

Speaking in a general way, all mitochondria have smooth and even outlines, no matter how bizarre their forms may be. Each merges into the other through imperceptible transitions, so that any terminology is more or less arbitrary. No difference can be detected in the solubilities or in the staining reactions of the different forms. A fundamental distinction may, however, be made between

variations in length and variations in breadth. While the mitochondria in the acinus cells of the pancreas, for instance, vary greatly in length, filamentous and rod-like forms predominating, their girth is remarkably uniform, throughout the whole tissue, in individual cells and in different parts of the same filament. The ends of the filaments are never tapering. They are quite abruptly but smoothly rounded off. Similarly in other tissues the diameter is fixed, but the length is not; one is stamped on the cell through its organization, the other is not. We have here two attributes, independently variable, which may perhaps be influenced in different ways.

Evidently mitochondria increase in size by growth in length, probably by the addition of material at their extremities. That is to say, when mitochondrial substance is added the extension is always lengthwise, never lateral. It is equally true that when foreign material, like starch, pigment, or fat is deposited within the mitochondria, expansion is always provided for by increase in girth. There must be some difference in the method of addition. At present no explanation is available.

Much confusion would have been avoided if investigators could have come to realize that the same material under different conditions may assume different forms. The unfortunate part about it is that the conditions are almost wholly unknown to us. But whatever they may be, it is altogether likely that they operate in much the same way in plants and animals, because all forms of mitochondria are represented in both.

We have abundant evidence that mitochondria are semi-fluid in consistency. They flow together and fuse to form large droplets, under certain conditions, and in the streaming protoplasm of plant-cells their form is continually changing in response to currents and eddies in the stream. This semi-fluidity, together with their lipid-like properties, is responsible for their remarkably smooth and even outlines and rounded ends. It precludes rough excrescences, sharp angles, and pointed ends.

The very pronounced property which they have of being able to take in substances from the surrounding cytoplasm and heap them up and concentrate them in their interior is responsible for many of the swellings and enlargements which occur in certain situations in the course of the filaments as well as in the smaller rods and granules.

It is hard to say how great a part osmotic factors play in molding the shape of mitochondria. The mitochondrial substance is sometimes denser in the peripheral parts of the filaments and larger granules and bears some semblance to a membrane. The Lewises (1915, p. 373) found that they could alter the form of mitochondria by changing the osmotic pressure of the fluid bathing the cells in their tissue cultures. With hypotonic solutions there was a marked increase in size, with hypertonic a decrease. The osmotic pressure of the blood is very constant and has been so, if Macallum's work is correct, for untold ages in evolution. Since the same blood bathes tissues containing entirely different mitochondria, it seems that the osmotic pressure of the fluid about the cells must play a very minor

rôle, if any, in determining the shape of the mitochondria in the living organism. The mere presence or absence of high osmotic tensions within the cells makes no difference, because in certain plants pressures as high as 13 atmospheres are developed, yet, as far as our observations go, the mitochondria differ in nowise from those in plant and animal cells at atmospheric pressure.

It is quite possible that variations in the acidity or alkalinity of their surroundings may affect their form. Bearing in mind the fact that the mitochondria are supposed to be a combination of lipoid and albumin, we might conceive of acidity acting upon their protein fraction, causing it to become hygroscopic and to swell. But we must remember that oxidized phospholipin has a much greater affinity for water than the unoxidized (Mathews, 1915, p. 98). It is possible, then, that with increase in oxidation mitochondria will take up water and swell. But acidity inhibits oxidation. It begins to look as if we had two antagonistic influences to deal with. Acidity may cause the protein fraction of the mitochondria, which is the smallest fraction, to take up water; but it will also prevent the oxidation of the phospholipin and prevent it from taking up water. So that, arguing along these lines, one would expect the behavior of mitochondria to depend upon the relative proportions of phospholipin and albumin, or (more properly speaking) of protein, in their composition. But here we meet with the same difficulty as in the case of osmotic pressure. The astonishing neutrality of the organism would prevent this factor, if it is one, from playing any great part.

We must simply acknowledge our ignorance and hope for the future. It may be said, however, that the shape and size of the cell have no influence upon the shape or size of the contained mitochondria; neither has the water-content or the consistency of the cytoplasm.

We may conclude by saying that variations in the shape and size of mitochondria constitute by far the most delicate criterion of cell injury known to us. Mitochondria react long before the nucleus, and their morphology is the first thing to change, though it is soon followed by alterations in distribution and in amount.

VI. DISTRIBUTION.

DISTRIBUTION IN ORGANISMS.

Mitochondria seem to be present in all organisms from man to the most lowly protozoon and from the angiosperms to the fungi, but their existence is doubtful in the myxomycetes, the schizomycetes, and most of the algae.

Some think that the mitochondrial substance is here present in solution, and this idea is supported by the staining reactions of certain bacteria. It occasionally happens that tissues prepared for mitochondria have been invaded by bacteria, in which case the bacteria stain just like the mitochondria by the Benda method, with iron hematoxylin and with fuchsin methyl green. I have found that large bacilli contain granules which stain intensely and apparently specifically with janus green. They resemble in distribution the so-called polar granules. Smaller forms often stain diffusely.

Others consider the mitochondrial substance to be really absent. According to them its function is carried on by the chloroplasts. The fact that mitochondria diminish in number progressively with the development of chloroplasts in the higher plants lends some support to this view. Mitochondria may even be entirely absent when the full complement of chloroplasts is attained.

We know of no other exceptions in the distribution of mitochondria, but it must be borne in mind that comparatively few organisms have been investigated as compared with the magnitude of the phylogenetic series and exceptions may be brought to light at any moment.

DISTRIBUTION IN TISSUES.

Mitochondria occur in all the tissues of both plants and animals, with few exceptions, wherever protoplasm is active; except, of course, in the plants already mentioned. They are found in epithelial tissues, muscular tissue, bone, and all others, except in the terminal stages of cytomorphosis; and this (according to N. H. Cowdry) is one of the greatest points of similarity between these granulations in the plant and animal kingdoms; that is to say, their progressive diminution and final absence in the later stages of the life of the cell. It will be discussed in detail on page 78.

DISTRIBUTION IN CELLS.

Within individual cells mitochondria are, in the vast majority of cases, distributed indifferently, without definite order, throughout the cytoplasm; but there are some notable exceptions, examples of which may be seen by reference to plate 1.

The distribution of mitochondria with respect to *polarity in secreting cells* in animals is most remarkable. In the acinus cells of the pancreas (plate 1, fig. 9) the mitochondria are most numerous in the basal region next the basement membrane. They are long and filamentous and are, in a general way, distributed parallel to the long axis of the cell, streaming from the basement membrane toward the lumen. A similar arrangement obtains in other glands like the vesicula seminalis

(plate 1, fig. 3). Here the polarity, or the direction of secretion, is proximo-distal—that is to say, from the basement membrane in the direction of the lumen—and is indicated by the arrow.

The mitochondria are, however, arranged entirely differently in the epithelial cells of the intestine (plate 1, fig. 10). Champy (1911, p. 109) emphasizes the fact that here they are sometimes gathered together at both poles of the cell, instead of being heaped up only in the basal part, as in the pancreas. He interprets this as meaning that the intestinal epithelial cells are polarized in two directions for secretion and absorption. This is again represented by the arrows.

In the thyroid (plate 1, fig. 8) mitochondria are not most abundant in the part of the cells next the basement membrane as in the pancreas, parotid, and other glands, but are much more numerous in the distal region of the cell next to the lumen and the colloid substance. Bensley (1916, p. 50), on other grounds, has concluded that the original proximo-distal polarity of the thyroid cells has been reversed; that the normal direction of secretion is toward the basement membrane, blood-vessels, and lymphatics, instead of in the direction of the intrafollicular colloid as has been generally supposed, and he points out that the mitochondria are also reversed.

To sum up, in all glands where the polarity is proximo-distal (parotid, plate 1, fig. 7), the mitochondria are accumulated in the proximal part of the cell; in cells with double polarity (intestinal epithelium, plate 1, fig. 10), the mitochondria are heaped up in the distal as well as in the proximal cytoplasm; and finally in cells with reversed polarity (thyroid, plate 1, fig. 8), the mitochondria are gathered together in the distal part of the cell instead of in the proximal.

In view of these facts one is tempted to inquire whether the clumping of the so-called bâtonnets of Heidenhain, which (according to Regaud) are derivatives of mitochondria, in the proximal parts of the cells of the kidney tubules (plate 1, fig. 1), means that the original proximo-distal polarity of these cells is maintained and that secretion takes place from the basement membrane toward the lumen.

The question is, how universal is this apparent relation between mitochondria and polarity? Are the mitochondria ever uniformly distributed in gland-cells which are polarized? And, conversely, are they ever arranged in this way in cells which are not polarized? It may be said that this accumulation of mitochondria in the basal parts of the cells occurs in all zymogenic cells with proximo-distal polarity which have been investigated in mammals. In addition to the examples already cited the following may be mentioned: the cells of Paneth of the small intestine, the chief cells of the stomach, the serous cells of Harder's gland, etc.

I know of no instance in which the mitochondria are uniformly distributed in definitely polarized gland-cells. It is certainly true that in gland-cells which are unpolarized, or are but slightly polarized, the mitochondria are more uniformly distributed throughout the cytoplasm. Thanks to the work of Nicolas, Regaud, and Favre (1912a, p. 201), we know the relations of mitochondria in human sebaceous glands where the cells themselves are bodily transformed into the secretion and are passed out through the ducts. The cells (particularly the older ones) well

along in the metamorphosis show no evidences of polarity and contain mitochondria scattered uniformly throughout the cytoplasmic area. In the thymus gland the cells are unpolarized and the mitochondria are not arranged in any definite way. The so-called "rhagiocrine" cells of Renaut are unpolarized and contain mitochondria quite uniformly distributed. Other instances might be cited. We must regard secretion as a fundamental property possessed by all cells. Where they specialize in it, and give up all their energies to it, and retain their primitive connections with the basement membrane, the polarity is the most pronounced and the arrangement of the mitochondria the most striking. It is hard to say whether, when cells become depolarized (if they ever do), the mitochondria lose their polarized arrangement and become distributed uniformly. This has a very definite bearing upon the problem of the relation of mitochondria to polarity in gland-cells. It would seem that the carcinomata would constitute favorable material, because in some of them the secreting cells lose in a measure their power to secrete and often with it their polarity and take on unusual powers of multiplication. At such times one would expect the mitochondria to become redistributed uniformly throughout the cytoplasm.

In plants secreting cells are not so generally polarized and the ones which are polarized have not been investigated, so that we can obtain no help in this direction.

Child's (1915, p. 202) view that, in general, polarity is dependent upon the axial gradient in metabolism, considered with the likelihood that mitochondria themselves probably play some part in metabolism, is perhaps not without significance. There may be some relation here, and there may not—we can not tell. Tashiro (1915, p. 112) has established, by measurements of the CO_2 output, a gradient in metabolism of the nerve-fiber which corresponds with the polarity of the nerve-cell. I have diligently searched in nerve-cells of many forms for some relation between the distribution of mitochondria and dynamic polarization and have failed to find any. The polarity of egg-cells—that is to say, the plane in which cleavage takes place—is apparently not related to the arrangement of mitochondria. Beekwith's (1914, p. 217) centrifuge experiments have shown that the plane cuts the mass of mitochondria at any angle, sometimes into very unequal parts, which is in full accord with Lillie's (1908, p. 907) previous work, showing that in the egg polarity is dependent upon the organization of the ground substance itself, not upon the arrangement of any visible granulations within it.

Before closing this subject it may be emphasized that, so far as I know, there is no valid reason for the general assumption that all forms of polarity, the polarity of secreting cells, the polarity of nerve and egg cell, etc., are referable to the same fundamental cause, which some are inclined to do. An axial gradient in metabolism and the distribution of mitochondria may be factors in one type (*i. e.*, in gland-cells) without having anything at all to do with the others. I am inclined to think, however, that the truly remarkable distribution of mitochondria in secreting cells is much more likely to be one of the results or manifestations of the polarity rather than the cause of it. It may be entirely a question of mechanics, the mitochondria being absent from the distal zone, owing to the pressure of secretion therein.

During cell division the mitochondria are distributed in much the same way in plants as in animals. They persist during the whole process; they are absent in the spindle area, whether a definite spindle be formed or not; and they are divided in approximately equal amounts between the two daughter-cells. But in minor respects their distribution varies more in animals. I have reference, for instance, to the mitochondrial palisade described by Benda (1902, p. 781) in *Blaps*, which is (so far as I know) unknown in plants. In animal cells they are almost invariably disposed in a radial fashion about the *centrosome*, but such a condition has, to the best of my knowledge, not been described in plants. This discrepancy may, however, be due to the well-known absence of a typical centrosome in the angiosperms. Kingsbury (1912, p. 45) makes the interesting suggestion that (since in the terms of Lillie's theory of cell division the centrosome is a negative center) the mitochondria, being reducing substances, carry a positive charge and accumulate around the centrosome in order to discharge it.

In the cells which are not dividing the mitochondria sometimes heap up about the centrosome and sometimes do not. Their behavior may indicate whether the centrosome is active.

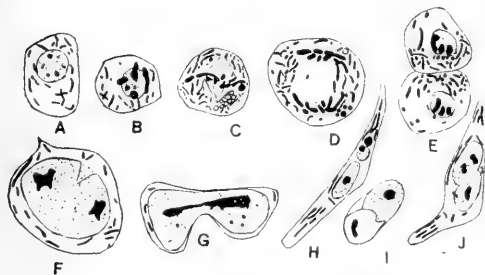


FIG. 1.—Dividing cells in chick embryo. A-E in mitosis and F-J in apparent amitosis.

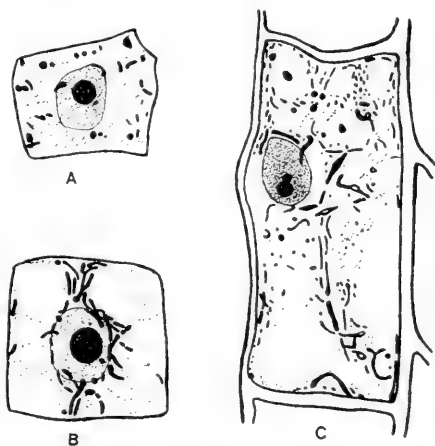


FIG. 2.—Meristem and young and old cortical cells of the pea, showing primary diffuse arrangement of mitochondria (a), secondary condensation about the nucleus (n), and final even distribution throughout the cytoplasm (c). (After N. H. Cowdry.)

Perinuclear condensations of mitochondria occur in both plants and animals. In the early meristem of plants, generally, mitochondria are found indifferently distributed in the protoplasm. They seem to approach and come in actual contact with the nucleus, in which position they enlarge and form plasts which migrate away from the nucleus and become distributed more or less evenly in the surrounding cytoplasm. Guilliermond has repeatedly described this migration and finds that the mitochondria become more and more resistant to acetic acid during this process of plast formation. Similarly, in the spermatogonia of certain animals the mitochondria make their way to the nucleus and become so closely applied to it that investigators have been deluded into thinking that they actually originate

from it. In the later stages of spermatogenesis they leave the nucleus, becoming more resistant to acetic acid, as Regaud (1910, p. 298) has shown. Indeed, the parallelism is very close (figs. 2 and 3). The Lewises (1915, p. 349) have observed mitochondria journeying to the nucleus and back again in the living cells of tissue cultures. Here their movements are much more rapid and no change in composition is evident. What their mission is we have no idea.

They also gather together in the *peripheral cytoplasm*, just beneath the cell-membrane, especially in animal cells. This arrangement is very pronounced in egg-cells and it has often been alluded to by Van der Stricht (1909, plate 1) and his pupils. It is a general phenomenon for which there must be some explanation. After a time the mitochondria become redistributed, just as in the perinuclear condensations. Curiously enough, other cells, like gland-cells, rarely if ever show it. An interesting reversal of this condition is seen in certain pathological conditions where the mitochondria quit the peripheral cytoplasm and become heaped up about the nucleus instead.

Both perinuclear condensations and peripheral condensations frequently occur in one and the same cell, as in the ascidian eggs described by Loyez (1909, p. 192).

In *ciliated epithelial cells* the mitochondria are often permanently heaped up in the region of the cytoplasm just beneath the ciliated border. I have found that this is the case in the ciliated cells of the epididymis of the white mouse. This fact, together with the familiar clumping of mitochondria about the axial filament in the tail of the spermatozoon and the feeling that they are transformed into myofibrils, led Benda to the conclusion that they play a part in the motor activities of the cell.

The *clumping and fusion* of mitochondria to form other substances is to be regarded as a very special instance of modifications in their arrangement. A discussion of this process logically falls under the heading of histogenesis. It may be simply mentioned here that we come across it in the formation of the nebenkern, the spiral filament, certain portions of the rods and cones of the retina, and other structures.

It is unnecessary to go into a discussion of other minor variations in the arrangement of mitochondria, dependent upon the deposition of substances in the cytoplasm, upon pressure, and other obvious causes.

In all these journeyings of mitochondria to and fro, and in these transitory and permanent condensations and fusions, not a shred of evidence can be seen that they possess powers of independent motility like bacteria. The prevalent belief that they do possess these powers seems to be simply a relic of the old conception of Altmann that they are elementary organisms endowed with all vital properties, just as the idea that in all cases they arise from other mitochondria by longitudinal or transverse division persists under the guise of the misleading doctrine of mito-

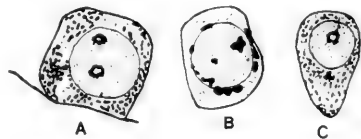


FIG. 3.—Spermatogonium, spermatocyte, and spermatid of mouse, showing (A) similar diffuse arrangement of mitochondria, (B) secondary circumnuclear condensation, (C) final even distribution in the cytoplasm. (After N. H. Cowdry, 1917.)

chondrial continuity. A very careful study made by N. H. Cowdry, in this laboratory, of mitochondria in the streaming protoplasm in many varieties of plant-cells (where they may be easily followed unstained) failed to reveal any indication of independent motility on their part. True, they dart from place to place in the cytoplasm, but this may in almost all cases be referred to definite currents and eddies in the stream. Moreover, I have examined mitochondria vitally stained with janus green in human polymorphonuclear neutrophile leucocytes during amoeboid movement and phagocytosis and I have likewise failed to detect any sign of independent motility. The movements can not, however, be entirely explained away on the basis of currents in the cytoplasm, because we have no reason to suppose that such currents exist in the egg-cells. They seem to be almost purposeful. They are entirely different from Brownian movement, though the mitochondria do exhibit true Brownian movements when the cells take up water and when the balancing action of bombarding molecules is upset. The consistency of the cytoplasm has little to do with it, because peripheral condensations occur in nerve-cells as well as in egg-cells; in nerve-cells the cytoplasm is very viscid, in egg-cells very fluid. The mitochondria may be easily thrown down with the centrifuge in egg-cells, but not in nerve-cells.

It may be a question of adsorption. The Gibbs-Thomson principle tells us that any process which diminishes free energy at an interface will tend to take place. Now, the mitochondria are nothing less than minute particles of lecithin-like material in suspension, and we know that lipoids decrease surface tension, so that we would naturally expect them to be heaped up at the nuclear and plasma membranes; but it is difficult to explain their active migration to and fro. Perhaps we are dealing with electrical adsorption. The mitochondria may carry a charge, but no adsorption could take place without the presence of a charge of the opposite sign upon the nuclear or plasma membrane, as the case may be, and of this we have no information whatever. Here again the movements backward and forward are the stumbling-block.

There is one more point for which a tentative explanation may be advanced. It will have been noticed "that in animal cells rather more variations seem to be met with in the arrangement of mitochondria than in plant cells. This may be correlated in some way with the fact that animal cells are more generally polarized; I mean polarized for irritability, conduction, secretion, contraction, and so forth, properties which do not play so great a rôle in the life of plants where separate regions of the cell are not so distinctly marked off."¹ The division of labor among animal cells is greater than in plants. They have to perform a great variety of functions under different conditions, so that in a single animal there is far greater diversity of organization among its cells than in a single plant. This the mitochondria reflect.

From a practical point of view it is a very simple matter, with comparatively little experience, to tell by the inspection of a cell whether the distribution of mitochondria within it differs from the normal, and this is another indicator of cell activity and of cell injury which has received but scant attention.

¹N. H. Cowdry, 1917, p. 215.

VII. AMOUNT.

AMOUNT OF MITOCHONDRIA IN PHYLOGENY.

What is true in the case of morphology holds also here. In man, as an example of a multicellular organism, with great division of labor among his cells, variations in amount of mitochondria occur because some cells are best fitted to perform their duties with much and others with little. From our general information we have no specific measurements—we can not say that the protoplasm of higher animals differs from that of the lower ones in the amount of contained mitochondria. Neither is there any noticeable difference in amount between animals and plants. Their apparent absence in the myxomycetes, schizomycetes, and most of the algæ has already been referred to on page 72.

AMOUNT OF MITOCHONDRIA IN ONTOGENY.

No definite measurements have been made. It may be said, however, that in the very young embryo the cells usually (p. 81) contain approximately the same amount of mitochondria. As development proceeds toward maturity the different tissues become specialized and distinctive differences in the amount of mitochondria often become apparent. It is possible, but improbable, that there is any noticeable difference in the number of mitochondria in actively functioning cells of young and old animals, but this contingency should be borne in mind until decisive information is forthcoming. There is no reason to believe that mitochondria vary in amount with sex.

AMOUNT OF MITOCHONDRIA IN CYTOMORPHOSIS (SENESCENCE).

Mitochondria progressively decrease in number and finally disappear entirely in the later stages of the life of the cell.



FIG. 4.—Meristem and parenchyma cells of the bean, showing the progressive disappearance of mitochondria and the formation of plastids containing chlorophyll. (After Guilliermond, 1912, modified.)

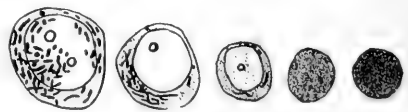


FIG. 5.—Erythroblast, megaloblast, normoblast, and erythrocytes from bone marrow of a rabbit stained supravivally with janus green, showing the parallelism between the disappearance of mitochondria and the appearance of hemoglobin in the form of a diffuse deposit. (After N. H. Cowdry, 1917.)

I refer to the decrease in number of mitochondria in plant cells which runs parallel to the formation of chloroplastids. It is said that when the plastids are fully formed few if any mitochondria remain (Guilliermond, 1912*a*, pl. 17), and these are mature and highly differentiated cells. In animals there is a similar disappearance of mitochondria in the life cycle of red blood-cells. In the young, nucleated forms, as they occur in the bone marrow, they are very abundant; but they become less and less so as the cell differentiates. A few persist after the

nucleus is lost, but in the senile forms, present in the circulation in man, mitochondria are entirely absent. In plants this disappearance is associated with the production of chlorophyll, in animals with the formation of hemoglobin, two substances with strikingly similar chemical constitution; in both it is gradual and progressive and runs parallel with an increase in the degree of differentiation and with the age of the cell, general metabolism being diminished and special functions being accentuated (see figs. 4 and 5).

This is but a single instance of a very widespread phenomenon which attracts attention only in those cells which normally die and are replaced in large numbers, collectively, in the life of the individual, like the cells of the epidermis. It is not without significance in any theory of *senescence*. Senescence is now thought to result from excessive differentiation with the heaping-up of relatively inert materials in the cell, which clog the vital processes and proportionally diminish greatly the volume of active cytoplasm. Child pictures these substances as large colloidal complexes, and Burrows (1917, p. 339) thinks that they are retained by virtue of their relative insolubility. Evidently, we have to deal also with a diminution in the mitochondria, but whether it is the cause or the result of the condition we can not tell.

AMOUNT OF MITOCHONDRIA IN DIVIDING CELLS.

It would appear that there must be some increase in mitochondria if the daughter-cells contain the normal amount and if their volume, taken together, exceeds that of the parent cell. Apparently, the increase is progressive and proportional to the volume of the cytoplasm. I have made (1914c, p. 102) a careful study of 1,000 dividing cells in chick embryos and at no stage in the process is it possible to say that the number of mitochondria in the cytoplasm is relatively greater than in the neighboring cells of the same type. This is confirmed by the Lewises (1915, p. 371).

Usually the mitochondria are distributed in approximately equal amounts to the two daughter-cells, but the distribution is a haphazard one, depending only upon the arrangement of mitochondria in the parent cell. An admirable instance of unequal division is to be found in the cleavage of the ascidian eggs as described by Duesberg (1917, p. 481). In my opinion the great differences in the amount of mitochondria in the tissues of the embryo are determined by the physiological condition (p. 82) of the cells rather than by whether or not many mitochondria had been handed down to them from some remote cell ancestor.

AMOUNT OF MITOCHONDRIA IN DIFFERENT CELL TYPES.

Striking variations in the amount of mitochondria obtain, but unhappily there has been but little attempt made to distinguish between absolute and relative variations. The obstacle is particularly exasperating in gland-cells, and this is just where the demand for information is most insistent, because the cyclical changes in the volume of secreting cells can not be ignored. In all tissues the estimation of mitochondria should be carefully controlled by measurements of

volumes, especially in experiments. This difficulty is almost insurmountable in muscle-cells, but it is comparatively negligible in nerve-cells.

Accordingly, Thurlow (1917, p. 37), working in this laboratory, selected nerve-cells for study. She managed to enumerate the mitochondria with surprising accuracy by inserting in the ocular a glass disk on which a square of known dimensions had been ruled. Using a 1.5 mm. apochromatic objective and sections of known thickness, the number of mitochondria per cubic millimeter of cytoplasm was easily calculated. Observations were confined to the cells of the nuclei of the cranial nerves, because they may be most readily referred to the different functional types. She carefully controlled her counts and found that the experimental error was never more than 1.3 per cent. She found that there is a constant number of mitochondria per unit volume of cytoplasm in normal nerve-cells of the corresponding cranial nerves of different white mice; further, that the constant differs for cranial nerves of different types, so that certain groups of nerve-cells can be distinguished by the number of mitochondria within them. The amount does not depend upon whether the cells are sensory or motor in character. A casual inspection of the sections showed at once that the mitochondria are most abundant in the cells of the mesencephalic nucleus of the fifth nerve and least numerous in the nucleus of the tenth nerve. The counts disclosed that there are 284,378,159 per cubic millimeter in the former and 178,210,313 per cubic millimeter in the latter. She examined all the cranial nerves, this being only a case in point.

It is highly desirable that these studies should be extended to other varieties of nerve-cells in the central and peripheral nervous systems, and the study of late embryos and young animals might tell us when these remarkable differences in number become first manifest, for we know that in undifferentiated nerve-cells the mitochondria are fairly uniform in number. We want, of course, to discover whether or not these differences in amount arise at the time of functional maturity; but whichever way it turns out we shall have obtained a much-needed clue to their significance. Furthermore, this work supplies us with a new criterion of nerve-cell changes which has the rare merit of being quantitative, and, as such, may well be compared with the nucleus cytoplasmic ratio which we owe primarily to Hertwig.

The quantity of mitochondria does not depend at all upon their form. They may be heaped up in the condition of granules, rods, or filaments. Similarly, when the mitochondrial content is reduced to a minimum either form may predominate.

It may be argued that these counts of mitochondria do not give us any real information about the cells in question, because tissues, kidneys especially, which

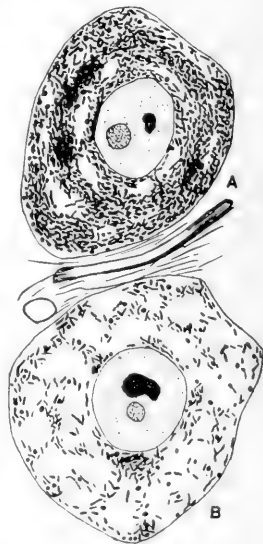


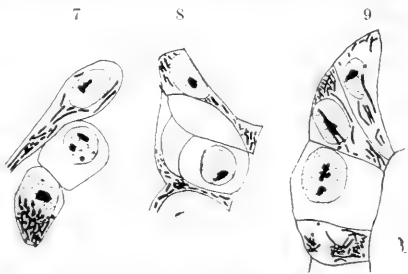
FIG. 6.—Spinal ganglion cells of the pigeon, showing (A) great increase in the amount of mitochondria as contrasted with the average amount (B).

show no evidence of fatty substances on histological examination may nevertheless actually contain a large amount as revealed by chemical analysis, and *vice versa*. The mitochondria are phosphatids, not neutral fat. It may also be urged that the cell contains a large variety of phosphatids of different solubilities and that our mitochondrial methods bring to light only those of a certain kind. This is undoubtedly true, but it is not the whole story, because our technique reveals all the phosphatids which occur in definite form and may be seen in the living cell, so that we are studying a constant, not a variable thing.

A word of caution in connection with the interpretation which we may justly place upon variations in the amount of mitochondria: In many pathological conditions there is a deposition of lipoids within the cell, the so-called lecithin metamorphosis. These must be carefully distinguished from true mitochondria. In other cases deposits of neutral fat occur which may be detected by the fact that they reduce osmic acid. Moreover, these substances almost invariably occur as spherules, very rarely as filaments, as is usually but not always the case with the mitochondria.

The total absence of mitochondria in normal actively functioning cells which are not senile (p. 79) is of considerable interest, though it is in all probability a phenomenon of very rare occurrence. Most of the examples recorded have, on further examination, proved to be erroneous; for instance, the parietal cells of the stomach, myeloblasts, the cells of malignant tumors, and others. I have never succeeded in finding mitochondria in tissue mast-cells, but I am inclined to think that this may simply be due to the acidophilic mitochondria being obscured by the densely packed basophilic granulations. They are present, however, in blood mast-cells. The cells in the kidney of snakes, described by Regaud (1908*a*, p. 17), and the cells of the glomeruli of embryonic human kidneys (Policard, 1912*c*, p. 442; 12*f*, p. 12) have never been found to contain mitochondria. Branca (1911, p. 559) has failed to find them in the inferior or germinative zone of hairs. Other cases of the apparent absence of mitochondria may be cited.

In neighboring cells of the same type, which usually contain approximately the same amount of mitochondria, we occasionally come across variations in amount from cell to cell which are difficult to explain. One cell sometimes contains mitochondria in tremendous excess of the normal amount present in the cells on either side of it. The difference may be so striking as to lead us to think at first sight that we are dealing with a different kind of cell altogether. In others there may be a decrease. The decrease is, I believe, often indicative of bad technique, just as we have learned to attribute to the same cause the artificial polymorphism of mitochondria in cells where they are normally alike. The increase I am unable



FIGS. 7, 8, and 9. —Neural tube, myotome, and Wolffian duct, showing cells apparently normal yet devoid of mitochondria.

to explain. It is very commonly met with in nerve-cells and in gland-cells (see fig. 6). In the cells of chick embryos I find that a decrease in the mitochondrial content is quite common (figs. 7, 8, and 9), but an increase above the normal very rare.

SIGNIFICANCE OF VARIATIONS IN THE AMOUNT OF MITOCHONDRIA.

We can say at once that such variations do mean something, because even if the mitochondria are as inert as iron filings their presence in such variable amounts must surely exercise some influence upon the activity of the cells containing them, and we have good reason to think that they are not chemically inert substances.

There are at least two series of observations to be explained. In the first place, the association of abundant mitochondria with intense protoplasmic activity. In cytomorphosis, for example, they are especially numerous in the active stages in the life of the cell and they diminish with senility in both plants and animals. There is a sharp increase in mitochondria with regenerative activity, in compensatory hypertrophy, and in many other conditions. In the second place, there is a distinct reciprocal relationship between the amount of mitochondria and the amount of fat. Where there are few mitochondria there is much fat, and *vice versa*. Decreased oxidation favors the accumulation of fat and increased oxidation favors its elimination, which suggests at once some connection between the amount of mitochondria and oxidation; and their abundance in the active stages of the life of the cell, where protoplasmic respiration is rapid, points to the same conclusion. Further evidence of a convincing nature has been detailed elsewhere (p. 134), and it seems probable that normal variations in the amount of mitochondria are in some way dependent on variations in the respiration of the cells containing them.

VIII. CHEMISTRY.

THE CONSTITUTION OF MITOCHONDRIA.

It is an interesting and rather unusual occurrence, in the study of mitochondria, for three independent lines of investigation to yield similar results, yet Regaud (1908*d*, p. 720), in the first place in the study of mammalian tissues, Fauré-Fremiet (1910*a*, p. 622), who worked on protozoa, and the botanist Löwshin (1913, p. 203; 1914, p. 269) have all arrived at the conclusion that mitochondria are, chemically, a combination of phospholipin and albumin, which, in itself, speaks very strongly in favor of the unity of the class of granules under consideration. The evidence is briefly this:

(1) Mitochondria are almost completely soluble in alcohol, chloroform, ether, and dilute acetic acid. They are rendered insoluble by chromization. They are not doubly refractile and they do not stain with either Sudan III or Scharlach R. They are only sometimes blackened with osmic acid.

(2) It is said that part of the mitochondrial substance is not soluble in these fat solvents and it is supposed that this portion is albumin (see also Bullard, 1916, p. 26), for formalin and bichromate, which are used as fixatives for mitochondria, are energetic coagulants of albumin. Millon's reagent is the only color-test for protein which can be satisfactorily applied to material in section (the xanthoproteic reaction may also be used, but it is less satisfactory because it is more destructive). I learn from Dr. R. R. Bensley that the mitochondria do not give a definitely positive Millon reaction in comparison with the strong Millon reaction which is given by such cytoplasmic structures as the zymogen granules. Even if there were a change in color in the mitochondria it might not be of sufficient intensity to be appreciated in filaments of such extreme fineness as mitochondria (0.2 micron in diameter) embedded in a colored cytoplasm. I have obtained no success with the xanthoproteic reaction. Mitochondria do not give any of the color reactions of polysaccharides.

(3) Artificial mitochondria have been made by Löwshin of lecithin in different salt and albumin solutions (resulting in the formation of lecithalbumin), which apparently present the same form and solubilities as true mitochondria. They form granules, rods, and filaments which, he claims, multiply by division. He embedded them in glycerin-gelatin, fixed them, and found that they stained in the usual way by the various mitochondrial methods.

(4) The temperature solubility of mitochondria may also be significant. It has been discovered by Policard (1912*d*, p. 229) in the case of animal tissues and by N. H. Cowdry (1917, p. 220) in plants that the mitochondria are soluble at a temperature from 48°C. to 50°C., while the other parts of the cells remain practically unaffected. Phosphatids have a low melting-point also.

(5) Apparently the specific gravity of mitochondria is somewhat greater than protoplasm (Fauré-Fremiet, 1913, p. 602). This is determined by the centrifuge method. If they are thrown down they are said to be of high specific gravity. If the protoplasm is in the physical condition of a "gel" rather than a "sol," as in

the nerve-cell, the distribution of the mitochondria is unaltered by centrifuging (Key¹). There is no reason to believe that the mitochondria themselves are different. At any rate, where the method is applicable (*i. e.*, in egg-cells) the mitochondria are heavier than protoplasm, in which respect they conform to what we know of phosphatids and differ sharply from oils and neutral fats, which rise to the surface and float instead of being thrown down.

(6) Mitochondria act as solutes for various substances. They are often pigmented and assume the most brilliant hues. Prenant (Asvandourova, 1913, p. 293) has actually styled them "chromochondria" on this account. This solution of other materials in mitochondria is particularly frequent in plant cells. It may or it may not be significant from the point of view of their constitution.

(7) There seems to be a certain correspondence between variations in the histological picture of mitochondria and the variations in the phospholipin content of the same organ on chemical analysis. Thus Mayer, Rathery, and Schaeffer (1914, p. 612) have been able to alter the mitochondria experimentally in liver-cells. In stages with more mitochondrial substance, chemical analysis showed an increase in phosphorized lipid; in stages with less, a diminution. Fauré-Fremiet (1912*b*, p. 347) has extracted from the ovaries and testes of *Ascaris* a phosphatid with properties identical with those of mitochondria in the cells of these organs.

(8) Russo (1912, p. 215) has apparently been able to increase the number of mitochondria in the oöcytes of the fowl by the injection of lecithin. R. Van der Stricht (1911, p. 435) found that there are two different kinds of eggs in the cat, one containing much vitellus and the other containing only a small amount; and, further, that, following intraperitoneal injections of lecithin, the relative number of female offspring increased noticeably. In the normal condition 62 per cent are males, while after treating in this way only 23 per cent are males. That is to say, the administration of lecithin increases the amount of deutoplasm in the eggs, increases the number of eggs with much deutoplasm as contrasted with those with small amount, and in this way increases the percentage of females in the offspring. While this is of great interest in the determination of sex, and will be discussed in that connection, it is also of importance as an indication of a possible relationship between the amount of mitochondria and the phosphatid lecithin. The researches carried on about the same time by Whitman, and subsequently by Riddle, in the determination of sex in pigeons, are in complete accord with these observations of R. Van der Stricht. Riddle (1916, p. 389), in summarizing the results of a long series of studies, points out that, in the first place, the eggs of late summer and autumn produce mostly females and that their yolks are larger than those of the spring, which give rise chiefly to males; and secondly, that old, "overworked" females tend to produce female offspring earlier and earlier in the season, and that this, also, is correlated with larger egg-yolks. His chemical analyses showed that the storage metabolism is higher and the water-content lower in these female-producing eggs than in those which give rise to males. The general conclusion, of course, is that sex is conditioned by variations in rate of metabolism, which is

¹Personal communication.

of interest when we remember that all our evidence points to the conclusion that mitochondria are concerned in metabolism. This work of Riddle seems to give us another chance to correlate the amount of mitochondria demonstrated histologically with the results of chemical analysis as well as with variations in physiological behavior. It must be borne in mind that the deutoplasm and yolk are not necessarily mitochondrial, but the substances out of which they are built up are phospholipins and resemble the mitochondria very closely in some important respects.

VARIATIONS IN CONSTITUTION.

In some *varieties* of cells the constitution of the mitochondria apparently differs, slightly but noticeably, from that of the mitochondria in other cells, though in cells of the same kind their composition is very constant. It was at first noticed, in a casual way, on the examination of preparations, that the mitochondria were occasionally preserved in one kind of cell and were lost, or imperfectly fixed, in others. While it is possible that this may be due to some difference in the cells themselves, it is far more likely that we are really dealing with a true variation in the solubility of the mitochondria. In the staining of fixed tissues, also, one occasionally meets with differences in the reactions of the mitochondria to the stain. Similarly, the mitochondria in different varieties of cells color with janus green somewhat differently, some more easily than others. Many investigators have also found that some mitochondria blacken or turn gray with osmic acid, although others do not. But observations such as these are unsatisfactory in the extreme, because they can not be controlled and because there is nothing quantitative about them.

We owe our first detailed information to Regaud (1910, p. 295), who very carefully compared the solubilities of mitochondria in the different cells of the testis with respect to acetic acid. He found that there is a progressive increase in their resistance to acetic acid as one passes from spermatogonia to spermatozoa. Some years later Nicholson (1916, p. 336) applied the same methods of technique to the central nervous system of the white mouse and found that there also the mitochondria in certain varieties of cells presented different and characteristic solubilities in acetic acid. This will have to be done in other organs and with a large variety of solvents before we shall be able to arrive at even a glimmering of the variations in the constitution of mitochondria which occur in different types of cells.

Variations in the constitution of mitochondria in the course of histogenesis are often quite pronounced. We have all had the experience that technique which gives good results when applied to adult tissues is frequently very disappointing for embryos. This may be due to factors other than a difference in the mitochondria—to changes in the water-content, for example. Here we have no detailed observations on the mitochondria themselves to fall back on; they are much needed. If we exclude the cases of the transformation of the mitochondria into other substances, I think that we may say that there are no very great differences between the mitochondria in these different stages of *histogenesis*. Cer-

tainly the variations are not so great as are those between the mitochondria in different varieties of cells. Fundamentally the problem is the same, because the variations between different types of cells arise throughout the course of histogenesis.

In *phylogenesis* also we have to deal with certain variations in the constitution of mitochondria, but here it is greater. The technique which we use has to be specially adapted to almost every form that we study. There is undoubtedly some difference in the mitochondria. It is not all a question of their surroundings. A case in point is that of *Planaria*, in which I have attempted, again and again, to identify the structures which Korotneff (1909, p. 1010) has described under the heading of "Mitochondria." I have found that they are satisfactorily fixed in mixtures like acetic sublimate, which destroy mitochondria in mammalian tissues; and, conversely, that the methods which I have been accustomed to use for higher forms fail completely. This experience has impressed upon me the danger which lies in arguments that since the mitochondria do something in one type of cell they must necessarily do it also in another.

N. H. Cowdry (1917, p. 217) has made the first detailed comparison of the microchemical reactions of plant and animal mitochondria. His general conclusion (p. 225) is as follows:

"We have every reason to suppose that their *chemical composition* is much the same in both plants and animals, but here our knowledge is for the most part supposition and inference, since direct chemical analyses are obviously out of the question. Their composition, as indicated by solubility with respect to acetic acid, heat, and other reagents, is certainly subject to similar variations in both."

We know very little of variations in the constitution of mitochondria in different physiological conditions, though they probably occur. The only reference known to me of work along this line is Policard's (1912*d*, p. 229) on the temperature solubility of mitochondria in kidney-cells, in which he makes the unqualified statement that the temperature solubility varies with the state of physiological activity. This should be confirmed.

In conclusion, we may say that slight variations do occur in the chemical constitution of mitochondria in different varieties of cells, in the course of histogenesis and phylogenesis, and in different physiological states, though we do not know their nature or extent. They are the exception rather than the rule, but they must nevertheless be kept in mind when we venture to argue by analogy.

REACTION TO JANUS GREEN.

Michaelis (1899, p. 565), while making a detailed study of the behavior and chemical nature of vital dyes, found that janus green stained certain filaments in gland-cells specifically. The janus green was obtained from the Farbwerke Hoechst Company. He called it diethylsaffraninazodimethylanilin, and gave it the formula shown in figure A. He was careful to emphasize that a slight alteration in the composition of the janus green alters the specificity of the stain, for he found

that dimethylsafraninazodimethylanilin did not stain the filaments specifically. He found that, on reduction of the dye in the tissue itself or in the test-tube, the dimethylanilin group splits off, leaving the red diethylsafranin. According to him the diethylsafranin formed in this way does not stain the filaments specifically.

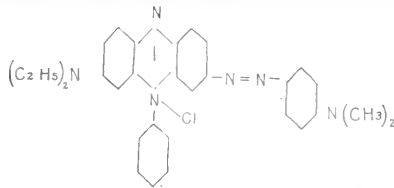


FIG. A.

Laguesse immediately recognized the importance of Michaelis's discovery, obtained some janus green from Grüber (not Hoechst), and used it in his investigations on gland-cells. It stained some structures which he called "vermicules" and "ergastidions." His results, however, were not uniformly satisfactory, probably on account of the fact that he obtained the dye from the wrong source.

Accordingly, janus green was soon forgotten as a vital stain for mitochondria, and it remained for Bensley (using the janus green of the Farbwerke Hoechst Company) (1911, p. 304) to revive interest in the discovery of Michaelis.

The reaction of mitochondria to janus green may be conveniently described in three stages: the staining of mitochondria with janus green, the reduction of the janus green with the formation of diethylsafranin, and the production of the leuco-base.

If one injects the pancreas of a guinea-pig through the blood-vessels with a solution of 1:25,000 janus green in 0.85 per cent sodium-chloride solution, the mitochondria become intensely stained in the course of 15 minutes or more. The other structures in the cell, like the nucleus and zymogen granules, remain uncolored unless the stain is applied in too great concentration for too long a time. The staining is facilitated by exposing the pancreas to the air.

If, now, portions of the pancreas are mounted in salt solution on a slide and are covered with a cover-glass, the dye is slowly reduced by the tissues, with the formation of diethylsafranin (see, however, Michaelis, 1902, p. 101), which has a bright pink color. The change first takes place in the more central parts of the tissue remote from the oxygen of the air and surrounding salt solution and proceeds slowly towards the periphery. It is hastened by ringing the preparation around the edge of the cover-glass with vaseline, which excludes the air and prevents evaporation. The faintly green-stained ground substance first changes to pink before the intensely stained mitochondria are affected. The change passes like a wave across the tissue from the center to the periphery. The mitochondria in the part of the cell nearest the middle of the preparation change their color first. Those in the remainder of the cell then follow suit. There is no evidence that mitochondria of different sizes change at a different rate, or that different parts of

the same mitochondrion differ in this respect. In this way the entire cell assumes a pink color, but the mitochondria are most intensely stained.

The next change is a further reduction of the diethylsafranin to the leucobase. The tissue bleaches. If the preparation is viewed with a low power, the periphery of the piece of tissue in contact with the surrounding fluid is colored green (stage 1); nearer the center a band of pink is seen (stage 2); the center itself is colorless; so that all stages in the reaction may be seen at one time. When the leucobase is once formed it is impossible to restrain the mitochondria with either diethylsafranin or janus green.

The reaction of mitochondria to janus green is essentially similar in other tissues. I have elsewhere (1914*b*, p. 276) described it in detail in human lymphocytes, which may be contrasted with pancreas-cells, since they float freely in a fluid medium.

All my attempts to make permanent preparations of these vitally stained mitochondria, made on the basis of the fact that both the picrates and molybdates of janus green are relatively insoluble in alcohol, proved futile.

Frozen sections may be made of the tissues stained with janus green, but the freezing generally brings about a destruction of the mitochondria.

Certain conditions may be recognized which tend to inhibit the janus-green reaction. The mucus that is secreted by *Planaria* and other invertebrates stains intensely with janus green, but the mucus secreted by leeches does not prevent staining of mitochondria in spermatogenesis. In this way a large amount of janus green is removed from the solution, with the result that the concentration of the solution in actual contact with the cells is much weaker than that originally applied. Moreover, masses of mucus often surround the cells and form barriers against the diffusion of the dye. Albumin acts in a somewhat similar way. When present in sufficient amounts, as in the blood and tissue juices of *Limulus*, *Callinectes*, etc., it becomes coagulated and stained by the janus green. It then presents a very confusing picture which is very likely to lead the unwary astray. A high concentration of sodium chloride (3 per cent) or potassium nitrate (3 per cent) will prevent the janus green from acting. The greatest obstacle, however, to the general use of janus green as a vital stain for mitochondria in all cells is its very limited power of penetration. For this reason the staining of mitochondria in intact brain-cells is very difficult. Here, however, another factor enters in, namely, the rapid reduction of the dye to its leucobases by the reducing action of the tissue itself, so that when the skull is opened up the brain is found to be colored red instead of blue. I have attempted to prevent this reduction by bubbling pure oxygen through the stain as it was being injected, without much success. It does not help matters to remove the skull-cap. Vasodilators are of no assistance in bringing a larger volume of solution in contact with the cells.

Conversely, other conditions favor the janus-green reaction. Of these, ready permeability of the cells is by far the most important. It is highly desirable that the janus green should be brought into immediate contact with the cells themselves. This indeed is the reason why the best results are always obtained in blood-

cells and in protozoa, and in organs which may be stained by the injection of janus green through the blood-vessels.

Janus green occasionally stains other structures in addition to mitochondria, of which a few may be enumerated: cement substance between the cells of the testis, lipid inclusions, blepharoplasts, chromosomes, granules of islet-cells, etc. But it is important to note that it is specific for mitochondria when used in great dilution, 1 : 500,000, for instance.

The toxicity of janus green varies in different cells of the same animal and in different animals. The same is true in plants. Fresh and old solutions of the dye have the same toxicity. Lewis found that in cultures of heart-muscle cells the mitochondria stained with janus green while the muscle continued to beat. Shipley (1916, p. 441) found that trypanosomes retained their motility for some time after the mitochondria in them were specifically stained with janus green.

I have been able to stain the mitochondria with janus green in the polymorphonuclear leucocytes of man and several other vertebrates. Such leucocytes, with their mitochondria stained, move around in an amœboid fashion. The tip of the pseudopod is generally free of both mitochondria and the specific granulations. It is followed by a mass of mitochondria and granules. The nucleus generally comes last. Cells stained in this way engulf foreign particles and show no deviations from their normal behavior. They will continue to do this for from half an hour to an hour, depending upon the temperature of the warm stage, the rate of evaporation, etc. Their rate of disintegration is not greatly accelerated by the action of the stain.

When applied subcutaneously it remains localized at the point of injection. Intraperitoneal injection causes death in a short time and intravenous injection in a very few minutes. Feeding experiments gave no results and attempts to increase the tolerance of protozoa to it proved futile.

To what is the toxicity due? There are a number of possibilities. It may be due to the presence of some toxic material used in the manufacture, but rather against this is the fact that different samples of janus green B possess the same toxicity when applied to the same type of cell. The toxicity varies, however, markedly in different kinds of cells, and it is not a question of penetration. The idea of obtaining, in some way, a completely non-toxic janus green is an inviting one, but janus green can never approximate to the azo dyes like trypan blue in this respect, for the reason that it quickly undergoes chemical change in the body, with the liberation of a number of potentially toxic substances.

Before taking up the specificity of the janus-green reaction, it will be of interest to bring out a few more facts about janus green itself.

The name may or may not be associated with the Roman deity Janus, generally represented with two faces looking in opposite directions. The dye certainly shows two colors, green and red, of quite opposite character.

According to Michaelis (1902, p. 51), janus green is made by the action of nitric acid on safranin, one of the two amido groups being changed into the diazo group. To this dimethylanilin is added according to the following equation:

Diazo-diethylsafranin

Dimethylanilin

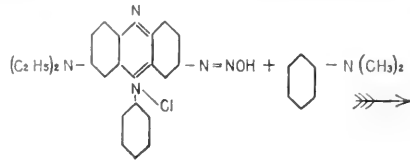


FIG. B.

Diazingrün (janus green):

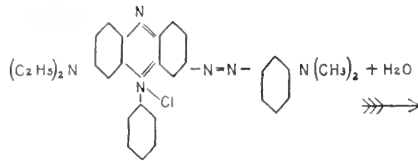


FIG. C.

Diethylsafraninazodimethylanilinchloride:

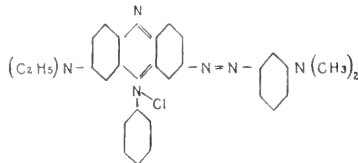


FIG. D.

This is the janus green B of the Farbwerke Hoechst Company. It is the same as "Diazingrün" and may be the same as "Halbvollgrün," but of this I am not certain. There are two other janus greens of different formulae, which makes three varieties of janus green in all:

(1) Janus green (Grübler), safraninazodimethylanilinechloride:

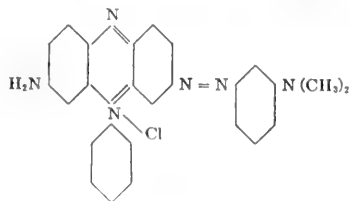


FIG. E.

(2) Janus green C (Farbwerke Hoechst Company), dimethylsafraninazodimethylanilinchloride:

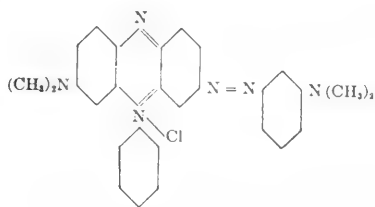


FIG. F.

(3) Janus green B (Farbwerke Hoechst Company), diethylsafraninazodimethylanilinchloride:

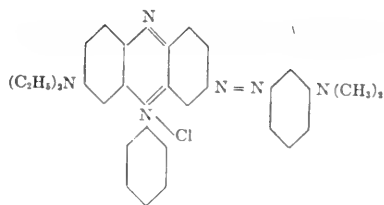


FIG. G.

The specificity of the reaction is shown by the fact that only the latter, janus green B, the one originally recommended by Michaelis, will stain mitochondria, though the others differ only in the substitution of an H_2 or $(CH_3)_2$ in the place of the $(C_2H_5)_2$ group. The presence of the diethyl group in the safranin molecule is evidently the determining factor. Compounds containing two ethyl groups are more basic than those containing two methyl groups, and the compounds containing two methyl groups are, in turn, more basic than those with two hydrogen atoms alone, so that there is a decrease in basicity as we pass from the diethyl to the dimethyl and to the dihydrogen. This may well explain the difference in the behavior of these janus greens toward mitochondria. One would expect to find that janus green G, with the two methyl groups, would color the mitochondria better than the janus green of Grüber, which possesses only the H_2 group, but I have failed to detect any difference between the two. Both of them occasionally stain mitochondria, together with other cell structures, when used in relatively high concentrations. Nevertheless, the difference in the actions of the janus green B with the two ethyl groups and the other two dyes, together with the difference in basicity between them, would suggest that the dye actually combines chemically with the mitochondria and that the staining is not simply a process of selective absorption. Our evidence, however, is too scanty to permit us to arrive at any conclusion. The poor results obtained with some samples of the janus green are probably due to admixtures of the first and second varieties.

The properties of janus green B are: (a) in water, blue solution; (b) on addition of hydrochloric acid, soluble blue precipitate; (c) on addition of caustic soda, black precipitate; (d) in concentrated sulphuric acid, olive-green solution, on dilution becoming green, then pure blue.

The azodimethylanilin has but little to do with the specificity of the reaction, because the diethylsafranin alone will stain the mitochondria more or less specifically. Moreover, I have prepared the safranin from the janus green of Grüber, the dimethylsafranin from the janus green G of Hoechst, and the diethylsafranin from the janus green B of the same firm, and I find that the diethylsafranin alone will stain the mitochondria.

The method of preparing the safranin is as follows:¹

1. Make a saturated solution of janus green in distilled water in a flask.
2. Add a little zinc dust and a few drops of hydrochloric acid. The solution first assumes a bright crimson color and then bleaches, the hydrochloride of the leucobase of the safranin being formed.
3. Filter. Shake the filtrate in air and thus reoxidize the leucobase.
4. Precipitate the dye by saturating the solution with sodium sulphate. It is often necessary to use a little heat. A dark red precipitate is formed.
5. Filter. Collect the precipitate on the filter. Wash with a saturated solution of sodium sulphate and dry it.
6. Dissolve out the dye from the dried precipitate with absolute alcohol.
7. Filter and evaporate the filtrate to dryness.
8. Dissolve the dye in the required concentration in distilled water or in salt solution.

The diethylsafranin prepared in this way behaves in exactly the same fashion as some pure diethyl safranin manufactured especially for me by the Farbwerke Hoechst Company.

There is, in addition to these janus greens, a large series of other janus dyes, of which janus blue G and R, janus gray B and BB, janus black D, I, II, and O, and janus yellow G and R are of particular interest because they are safranin derivatives, the others being dyes of other series.

Janus blue is diethylsafranin-B-naphthol and it stains mitochondria in living lymphocytes in a constant and specific fashion. It is inferior to janus green in that it will stain mitochondria in these cells only in a dilution of 1:300,000, but as an indicator of processes of reduction it is better than janus green, for the contrast between the blue of the dye itself and its red safranin base is more brilliant than in the case of janus green. The marks G and R indicate, according to Schultz (1914, p. 48), that the janus blue is made by two processes, from elematin (mark G) and from safranin (mark R). It is worthy of note that this color contrast with janus blue is particularly beautiful in the kidney, where the glomeruli may be colored deep blue and the remainder of the tissue red; so sharp is the contrast that the glomeruli in thick sections of the entire kidney may be easily counted with a binocular. Janus green likewise stains the glomeruli specifically.

Janus black I also stains mitochondria in living blood-cells specifically, but, on examination, I find that it is not a pure dye, but a mixture of two substances, diethylsafraninazodimethylanilin and a brown substance, the nature of which I

¹Dr. R. R. Bensley, personal communication.

am unable to determine. Thus, the specificity of janus black is undoubtedly due to the fact that it contains janus green as one of its ingredients.

I have isolated the diethylsaffranin from janus blue, janus black, and janus gray (I failed with janus yellow, which may not contain it), and they all stain mitochondria, which is further evidence that the specificity of janus green depends upon the diethylsaffranin group. It may be said that the staining is favored by the addition of azodimethylanilin to it, as in janus green; increased, though not so much so, by adding B-naphthol; and altogether prevented by the addition of other groups, as in janus gray.

I have made an attempt to compare the specificity of janus green for mitochondria with other dyes which investigators have made use of for the purpose of staining them. I used living human lymphocytes in freshly drawn blood as material. The results of the comparison are shown in table 3. The names of the dyes are given in the left-hand column, the concentrations are noted above, and a few notes are recorded on the right. Intense staining is designated #, a faint coloration +, while the minus sign indicates that the mitochondria are entirely unaffected.

TABLE 3.—Specification of vital stains.

	1:100	1:10,000	1:100,000	1:200,000	1:300,000	1:400,000	1:500,000	1:600,000	1:700,000	Remarks.
Janus green B (Hoechst).....	w	w	w	w	w	w	+	(?)	-	Gives a specific stain of mitochondria only.
Janus blue (Hoechst).....	w	w	w	+	-					Acts in the same way but rather more slowly.
Janus black I (Hoechst).....	w	w	w	(?)	-					Same as janus green B.
Diethylsaffranin (Hoechst).....	w	+	+							Stains mitochondria intensely and nuclei diffusely.
Nilblau B extra.....		+	+	+						Stains specific granulations more intensely than mitochondria.
Janus green G (Hoechst).....		+	+	+						Much the same as Nilblau B extra.
Janus green (Grübler).....		+	+							Tinges mitochondria only.
Methylene blue med. (Hoechst).....		+	+							Do.
Pyronin (Grübler).....		+	+							Do.
Bismarek brown (Grübler).....	+									Same, with diffuse coloration of entire cell.
Methyl violet 5B (Grübler).....		+								Tinges mitochondria slightly.
Brilliant Kresylblau (Grübler).....		-	-	-						Negative.
Dahlia (Grübler).....		-	-							Do.
Gentian violet (Grübler).....		-	-							Do.
Pyrrrolblau (Grübler).....		-	-							Do.

IX. EMBRYOLOGY.

FERTILIZATION.

Investigations on the behavior of mitochondria during the process of fertilization are at the root of the theory that they constitute the cytoplasmic basis of heredity. These studies have brought to light several facts:

(1) That in the multiplication of spermatogonia and spermatocytes in the testis, the mitochondria are divided in approximately equal amounts between the daughter-cells.

The mitochondria are usually divided en masse. There seems to be no general provision for a qualitatively equal division of the mitochondrial substance in any way analogous to the longitudinal splitting of the chromosomes. Wilson's (1916, p. 539) discovery of the peculiar behavior of the mitochondria in the scorpion must be mentioned. He found that in the Arizona scorpion there is in spermatogenesis an accurate qualitative distribution of mitochondrial substance. The mitochondria become condensed into a ring of material which "divides somewhat after the fashion of a heterotype chromosome ring, each spermatid receiving exactly one-fourth of its substance." In the California scorpion, on the other hand, the process is entirely different. "The ring is here absent, its place being taken by about twenty-four separate hollow spheroidal bodies that show no evidence of division at any time and establish no definite relation to the spindle, but are passively segregated by the spermatocyte divisions into four approximately equal groups. Each spermatid thus receives as a rule six, not uncommonly five, rarely seven of these bodies." Now, the astonishing regularity of the process in the Arizona scorpion recalls to mind the behavior of the chromosomes themselves and strongly suggests that we are here dealing with a distribution of substances which may actually play a part in heredity. But the phenomena observed in the California scorpion lead irresistibly to a diametrically opposed conclusion, that the mitochondria are not carriers of heredity, since their distribution to the spermatids seems to take place in an irregular, haphazard way. One can not help remarking how fortunate it is that the two forms were studied at once, because otherwise it would be difficult indeed to resist being led astray by the extraordinary behavior of the mitochondria in the first of them.

(2) That structures derived from mitochondria persist in the fully developed spermatozoon.

Benda (1897, p. 401) was the first to show that they become transformed into the spiral filament of the sperm. This observation has been corroborated by many authors. The mitochondria also form the "nebenkern." But the mitochondrial substance is usually confined to the middle piece of the sperm. Here also the great variations in the behavior of the mitochondrial material are recorded, and we notice at once the absence of anything approximating the remarkable orderliness which is so characteristic of the changes which the chromatin undergoes. Finally, the

fact has not been given due emphasis, so far as I am aware, that this mitochondrial substance which persists in the fully developed spermatozoon is very different from the typical mitochondria occurring elsewhere. It is well known that the mitochondria undergo definite chemical changes in the course of spermatogenesis. Regaud (1910, p. 294) clearly showed that their resistance to acetic acid grows greater and greater; in fact, the structures which they form, *nebenkern* and spiral filament, were known and recognized long before the mitochondria in the earlier stages of spermatogenesis were brought to light. They can be stained by ordinary methods of technique and are resistant to acetic acid. While it can not be denied that these structures are actually developed from mitochondria, it certainly requires a considerable stretch of our definition of mitochondria to include them under the heading of mitochondrial apparatus and to make the statement that mitochondrial substance occurs in the fully developed spermatozoon.

(3) That in many cases these mitochondrial products enter the egg on fertilization.

The observations of Meves (1911*b*, p. 709) on *Ascaris megalcephala*, confirmed by Held (1912, p. 247), Romeis (1913*b*, p. 166), and Fauré-Fremiet (1913, p. 585); those of Meves on *Parechinus miliaris* (1912, p. 102), *Phallusia mamillata* (1913, p. 225), *Filaria papillosa* (1915*b*, p. 58), and *Mytilus edulis* (1915*c*, p. 54); those of Duesberg (1915, p. 41) on *Ciona intestinalis*; of Levi (1915, p. 488) on *Vespertilio murinus*, and many others all show that this occurs.

It is to be noted, furthermore, what a very comprehensive list of animals has been studied which is representative of many of the great divisions of the animal kingdom. It is indeed a body of evidence which can not be well evaded. A few years ago derivatives of mitochondria were known to pass into the egg in only a few instances. Now we realize that they usually do so.

(4) That these same mitochondrial products which enter the egg in this way can be recognized up to a certain stage in the development of the resulting embryo.

Thus Meves (1912, p. 116) followed them through the first and second divisions of the fertilized egg. Fauré-Fremiet (1913, p. 586) studied the fate of the male mitochondria in *Ascaris*. Levi (1915, p. 523) traced them into a 3-blastomere stage in the Fallopian tube. It can easily be seen by a careful study of the illustrations presented by these authors, and it is important to note, that these substances behave in every way like foreign bodies; they undergo no changes and seem to exercise no influence upon the behavior of the other formed elements in the cytoplasm. In this they may be contrasted sharply with chromatin. It would be interesting to compare, with respect to mitochondria, the subsequent development of eggs fertilized artificially with some fertilized with sperm in the usual way. Jacques Loeb (1916, p. 316) has succeeded in rearing to sexual maturity frog embryos which were artificially fertilized. An examination of mitochondria during spermatogenesis in these forms might afford the most valuable information. If the mitochondrial content of the sperms differed from those of ordinary frogs it would be of the greatest significance from the point of view of whether or not the mitochondria are heredity carriers, which is the central theme of most of the recent studies on mitochondria.

INHERITANCE.

The doctrine that the mitochondria play a part in the transmission of hereditary traits is based upon the necessity of admitting the existence of a cytoplasmic heredity. The claim is based on the well-known experiment of Godlewski, which showed that an egg (deprived of its nucleus) when fertilized with sperm of another species retained certain maternal characteristics on development. In fact, there is nothing new in the conception that there is such a thing as cytoplasmic heredity. Jenkinson (1914, p. 152) and Conklin (1915, p. 176) freely admit it. What is new is the view that mitochondria carry it.

Let us pause a moment in order to consider the claims of the advocates of this view, which was first enunciated by Meves (1908, p. 849), though Van der Stricht also guardedly made reference to the possibility that mitochondria may play a part in heredity in 1908 (p. 4). They do not say or even hint (as the adherents of the chromosome hypothesis have not refrained from doing, in the case of the chromosomes) that the mitochondria constitute in any sense of the term the sole basis of heredity. They believe, however, in general, that the mitochondria play a part in the transmission of those characters which are cytoplasmic. Meves (1908, p. 850) says that the nuclear characters are carried over by the chromosomes, those of the plasma by the chondriosomes. This idea is beautifully supplemented by Meves's subsidiary hypothesis, according to which the mitochondria are transformed into all products of cellular differentiation. In other words, here is a material that can be seen to go through all the stages of development and actually give rise to the peculiar and characteristic differentiations which are the hereditary traits. The trouble is that the evidence that the mitochondria play a part in histogenesis (p. 102) is no more convincing than that in favor of the view that they constitute the material basis of heredity. Broman's working hypothesis is that while the chromosomes are the bearers of the hereditary qualities of the race and species, the mitochondria carry those peculiar to the individual. It is interesting to observe that Wilson (1914, p. 352) writes:

"Genetic experiment has already given some ground for the conclusion that definite types of hereditary distribution may be immediately dependent upon elements contained in the protoplasm. Recent advances in our knowledge of the 'chondriosomes' or 'plastosomes' provide this conclusion with at least a possible cytological basis."

All through the work on heredity, as well as in the other biological problems, the tendency is observed to affix the responsibility for the phenomena to structures with definite form—with each new discovery of a morphological entity to become optimistic and to think that we are well on the road toward a solution of the problem. The basic laws of heredity were appreciated by Mendel long before the discovery of the chromosome; yet the chromosomes were seized upon with great avidity. So it is with the mitochondria.

The adherents of the chromosome hypothesis in this country and elsewhere are naturally opposed to this view that the mitochondria are even partial carriers of heredity. It is said that the cases in which it has been shown that mitochondria

pass into the egg on fertilization are exceptional and that the crucial cases are those in which no mitochondrial substance passes into the egg. This Lillie believes to be the case in *Nereis*. Mitochondria generally occur in the middle piece and tail of the spermatozoon, though this is not always true. Lillie (1912, p. 418) says that "the middle piece and tail of the spermatozoon do not enter in the fertilization of *Nereis*." He admits (p. 426) that "it is possible that the fixation granules produced by the spermatozoon represent a cytoplasmic element." So that, until new facts are discovered, through the use of mitochondrial methods of technique, the case of *Nereis* does not offer an insurmountable barrier to the acceptance of the view that mitochondria play a part in inheritance.

MITOCHONDRIAL CONTINUITY.

The question of mitochondrial continuity arises just as surely as the doctrine of the permanence of the chromosomes, and the proof of it is every bit as unsatisfactory, perhaps more so.

Duesberg (1912, p. 766) is in favor of the theory of mitochondrial continuity; Beckwith (1914, p. 230), Chambers (1915, p. 291), and several others are against it. It has taken strong hold on the botanists, Guilliermond (1912*a*, p. 398) coming out strongly in favor of it and saying: "The mitochondria result from the division of the preëxisting mitochondria of the egg, none of them ever arise '*de novo*' in the cytoplasm." However, in certain plants some mitochondria are large and others extraordinarily minute. It is possible that the large ones may arise in this way by division of preëxisting ones, but there is no evidence that the small ones are formed only by the segmentation of filaments or rods, which are of larger girth than they are. They probably arise *de novo* by condensation.

The idea that all mitochondria arise from preëxisting mitochondria by division is a relic of Altmann's (1894, p. 155) doctrine, *Omne granulum ex granulo*, and has persisted in our minds on account of the newly conceived idea that mitochondria are concerned in heredity. Altmann thought that they were elementary organisms endowed with a certain measure of individuality. It is possible that the truly remarkable morphological resemblance which they bear to bacteria led him to believe that they multiply in this way. This conclusion has been supported quite recently by the growing tendency to regard mitochondria as plast-like in nature. It is apparently quite true that mitochondria form plastids in some plants, and, in view of the evidence at hand that the plastids exhibit a true genetic continuity, some of them always arising from preëxisting plastids by division, it was quite natural to assume that the mitochondria themselves behaved in somewhat the same way.

It has been generally believed for some time past that mitochondria multiply by transverse division and perhaps also by longitudinal division. The evidence for the latter method is meager and unsatisfactory and does not merit discussion. With regard to transverse division, it must be said that this undoubtedly does occur in some cases—for example, in the course of spermatogenesis of *Vespa crabro*, as was found by Meves and Duesberg (1908, fig. 39). This has already been men-

tioned. Fauré-Fremiet (1910*a*, p. 527) has also forwarded strong evidence that the mitochondria in certain infusoria multiply in this way. The multiplication of mitochondria in mammalian tissues is a phenomenon exceedingly difficult to demonstrate. As a possible source of error it should be borne in mind that the most common reaction on the part of filamentous mitochondria to unusual conditions is to fragment—that is to say, to multiply by transverse division. This is a very definite reaction to injury, by no means a normal method of multiplication. Again, in sectioned material it is impossible to say with certainty whether the appearances observed mean an actual multiplication by division or simply an approximation of originally separate mitochondria, for mitochondria everywhere show a tendency to clump together which is markedly enhanced if the tissue is in any way injured. The only way to obtain definite information on the subject is to study a process, rather than a process killed at a certain point, as in fixed material, or even a perverted process, as in living cells teased out and growing in isotonic media.

As against the doctrine of mitochondrial continuity, we have certain cells in which the mitochondria appear to go into solution. I have found that this is the case in the chromophile cells of the nervous system (Cowdry, 1916*b*, p. 41). There have been, so far as I am aware, but few observations (Chambers, 1915, p. 291) of a *de novo* origin of mitochondria in the cytoplasm, but to my mind the burden of proof is not that it occurs, but rather that it does not take place. It would surely be arbitrary to assert that the phosphatid albumin complex (of which mitochondria consist) always occurs in certain aggregates of definite size which are visible with our present powers of the microscope and that these aggregates multiply by division like independent organisms. In this connection we can only speculate, but it certainly seems much more likely that the phosphatid may be deposited free in the cytoplasm or upon an albuminous matrix, and that it subsequently grows by accretion. It is quite possible that when it attains a certain size or shape it divides for purely physical reasons; but in the absence of definite proof, one way or the other, it seems to me highly probable that mitochondria are continually arising in the cytoplasm *de novo*, and furthermore that perhaps this is the most important method of multiplication.

It is, however, incumbent upon those who believe that the mitochondria are carriers of heredity to demonstrate their continuity; and, further, the origin of mitochondria *de novo*, if it does take place, would be very difficult to reconcile with the view that they transmit hereditary traits.

The observations of Beekwith (1914, p. 216) on the eggs of *Hydractinea echinata* are of particular interest in this connection. She found that the cytoplasmic contents of the fertilized eggs on centrifuging separated into three layers—a layer of oil, a clear zone, and a layer containing mitochondria and vitellus. The first plane of segmentation cut these layers in various directions, resulting in planulas of very different appearance. Beekwith separated the two first blastomeres of centrifuged eggs and allowed them to develop. Some of these formed planulas, which,

though they contained no mitochondria, nevertheless appeared normal, from which she concludes that mitochondria are not necessary for differentiation. Duesberg (1915, p. 67) very aptly remarks that these experiments do not show that mitochondria do not play a rôle in the differentiation of tissues, because no tissues at this stage have yet become differentiated. He is not inclined to accept Beekwith's statement that mitochondria are absent in the early stages of oögenesis and that they do not appear until the vitellus is well formed (they form, Beekwith believes, *de novo* in the cytoplasm), which is totally at variance with all we think we know of the mitochondria in oögenesis, as well as being in contradiction to the observations of Tsukaguchi (1914, p. 117) on *Aurilia aurita*, an animal belonging to the same class as *Hydractinea echinata*.

The greatest obstacle to the acceptance of the view propounded by Benda and Meves is our conception of the chemical nature of mitochondria. If it is true that they are phospholipins it is hard to regard them as carriers of heredity, even though they may contain albumin also. It can not be denied that, chemically, chromatin appears to be the best fitted to play the part of heredity carrier. The relative equality in the amount of chromatin between the male and female gametes and the deficiency in the amount of the cytoplasm must mean something. Even should it be shown that mitochondrial substance passes over in fertilization in all animals, it may indicate nothing more than that a living portion of the sperm, capable of metabolism, enters the egg. It is a mistake, however, to arrive at a hasty conclusion, because those who make the conservative statement that mitochondria play some part in heredity occupy just as secure a position as those, on the other hand, who claim that chromatin is the sole heredity carrier.

In the higher plants it is well known that all the cells of the gametophyte contain the x number of chromosomes and the cells of the sporophyte, or sexless generation, contain the double number $2x$, yet no distinction has been shown in the mitochondria, which appear similar in every particular. Furthermore, in the aphides, or plant lice, there is also an alternation of generations, but a comparison of the mitochondria in the two has not been made.

ORGAN-FORMING SUBSTANCES.

Recent investigations on mitochondria in the early stages of the development of *Ascaris* (Fauré-Fremiet, 1913, p. 676) and of ascidians (Duesberg, 1915, p. 66) throw a flood of new light upon our conception of the so-called "organ-forming substances" (Conklin, 1905, p. 216).

Conklin (1905, p. 211) has discovered the fact that the cytoplasm of the egg of *Cynthia* is structurally differentiated into three substances—a clear substance, a yellow substance containing yellow pigment, and a gray substance containing yolk; and that "the upper clear half of the egg gives rise to ectoderm; the crescent of yellow protoplasm surrounds the posterior side of the egg just below the equator and is later transformed into the muscle and mesenchyme of the larva; the gray protoplasm occupies the remainder of the lower hemisphere and gives

rise to the endoderm, to the chorda, and to the neural plate." So much for the fate of the "ectoplasm," the "mesoplasm," and the "endoplasm," as he styles them. That these substances form the organs in question Conklin (p. 217) has shown beyond the shadow of a doubt. In the absence of one of the substances, the organ to which it would naturally give rise is not produced; conversely, each substance develops, if it develops at all, into the parts which it would normally produce. The portions of the egg which lack these substances form embryos which lack the corresponding organs. From these three fundamental substances he derives six (p. 218), viz, ectoplasm, endoplasm, myoplasm, chymoplasm, caudal chymoplasm, and chordaneuroplasm.

Duesberg (1915, p. 35) attacked the same problem in *Ciona* with new methods which revealed the mitochondria and which showed that they occur in very different amounts in the areas of cytoplasm described by Conklin. In the light of his work it is evident that the myoplasm of Conklin is simply an accumulation of mitochondria, the gray protoplasm a region whose vitelline granules are particularly numerous, and, lastly, the clear protoplasm nothing else than the fundamental ground-substance of the egg, which contains but few mitochondria. Duesberg's observations confirm the discoveries of Conklin as well as extend them. He found that the areas form just exactly the organs which Conklin affirmed—that the yellow crescent which contains many mitochondria forms muscle, etc. But he does not agree with Conklin's interpretation. To repeat, Conklin believes (p. 211) that "all the principal organs of the larva in their definitive positions and proportions are here marked out in the two-cell stage by distinct kinds of protoplasm." Duesberg (p. 60), on the other hand, is of the opinion that the different substances in which Conklin believes do not exist. "The different appearances of different regions of the egg and of the blastomeres depend not upon the existence of special substances, but upon a special distribution of the elements figured in the ovoplasm," that is to say, of mitochondria, vitellus, pigment, etc. In other words, the regions differ only in containing different proportions of the same substances; none of them possesses a special kind of substance. This interpretation, which I thoroughly believe in, is also in accord with Conklin's own observations. He writes (p. 212):

"Although these different oöplasmic substances are chiefly localized in certain regions of the egg, which give rise to certain portions of the embryo, this segregation is not quite complete. Most of the clear protoplasm is found in the upper (ectodermal) half of the egg, but some of it is also present in the lower half. Most of the yolk is found in the lower (endodermal) half of the egg, but a little of it is found in the upper half. Almost all of the yellow protoplasm is located in the mesodermal crescent, but a very small amount of it is found around the nuclei of all the cells. Thus samples of these egg-substances are contained in all the cells; nevertheless the segregation is so nearly complete that the clear, the gray, the light gray and the yellow areas are marked out with the greatest distinctness."

Apparently there is a distinct relationship between the amount of mitochondria and the amount of vitellus. For instance, the mitochondria are tremendously abundant in muscle-cells and the vitelline granules few in number; in nerve-cells a fair amount of both is present; while in the endodermal cells there is an enormous

amount of vitellus and few if any mitochondria. A similar quantitative relation between mitochondria and fat has been noted by many authors, and I have particularly in mind the observations of Goetsch (p. 136). We know that decrease in oxidation causes the deposition of fat and we suspect that mitochondria are actively concerned in oxidation (p. 134). It is common knowledge that they are particularly abundant in active cells, which must be respiring at a rapid rate. In other words, the heaping-up of fat and the diminution in the mitochondria indicate reduced oxidation; and conversely, the absence of fat and the abundance of mitochondria indicate an increase. Accordingly it is possible that the distribution of these substances, as described by Conklin and Duesberg, is merely the visible manifestation of differences in the rate of oxidation in different parts of the egg and in the different tissues of the embryo.

HISTOGENESIS.

The origin of the idea that mitochondria are concerned with histogenesis is not difficult to trace. They occur in all embryonic cells. In early stages of development they are the only formed elements in the cytoplasm. They are filamentous in the myoblasts and neuroblasts, and it is perfectly natural to think that they become transformed into fibrils and other products of differentiation; but the trouble is that the ways of nature are not simple, that the obvious interpretation is not necessarily the correct one. It also falls in line with the view that they constitute the material basis of heredity (p. 98). Meves (1908, p. 845) writes that, with the specialization of the embryo into different organs and tissues, primitively similar cells assume special functions which find expression in characteristic structures or differentiations. All these products, no matter how heterogeneous they may be, arise through the metamorphosis of one and the same elementary plasma-constituent, the chondriosomes. This is a very sweeping statement, but even it does not express the situation correctly, because claims are also made that most of the products of the activity of specialized cells of the adult organism, like secretion granules, are also formed by a chemical transformation of mitochondria.

The dominating influence of this dogma has made itself felt in many ways. To cite a single instance, Hoven in 1910 arrived at the conclusion that mitochondria are transformed into neurofibrils in the developing nerve-cell. This was generally accepted (Firket, 1911, p. 545; G. Arnold, 1912*a*, p. 289, and others). Following this line of reasoning, Hoven, (1910*a*, p. 478) and Meves (1910*b*, p. 655) concluded that the mitochondria are absent in adult nerve-cells after neurofibrillar formation has ceased, and looked for them and failed to find them. And this in spite of the fact that Altmann (1890, p. 52), Levi (1896, p. 180), Lobenhoffer (1906, p. 491), Nageotte (1909, p. 827), and others had already clearly and precisely figured and described them.

In order to get a true conception and perspective of what these claims really mean, I have tabulated some of the structures which are said to be developed through the transformation of mitochondria or under their influence.

List of substances supposed to be formed from mitochondria.

- Amyloplasts (= leucoplasts).
 Anthocyanin, Guillaiermond (1913c, p. 478; 1913f, p. 1002).
 Apparato reticolare (Binnennetz), Hoven (1910a, p. 479).
 Autoplasts (= chromoplasts).
 Aqueous humor, Mawas (1909, p. 284).
 Bâtonnets of Heidenhain, Policard (1912c, p. 457).
 Carotine, Guillaiermond (1912a, p. 389).
 Cerebrospinal fluid, Grynfeltt and Euzière (1912, p. 64).
 Chloroleucites (= chloroplasts).
 Chlorophyll, Guillaiermond (1912a, p. 407).
 Chloroplasts, Guillaiermond (1912a, p. 412).
 Chromoplasts, Guillaiermond (1912a, p. 412).
 Ciliary apparatus, Saguchi (1917, p. 266).
 Collagenic fibrils, Meves (1910a, p. 164).
 Composés phenolique, Guillaiermond (1913f, p. 1002).
 Connective-tissue cell granulations, Renaut and Dubreuil (1906b, p. 230).
 Cuticular formations, Van der Stricht (1918, p. 36).
 Cyanoplasts, Guillaiermond (1913i, p. 1282).
 Disque Q, Fauré-Fremiet, Mayer and Schaeffer (1910, p. 75).
 Eberth's intracellular structures, Saguchi (1913, p. 239).
 Elaioplasts, Guillaiermond (1912a, p. 405).
 Eosinophile granulations, Meves (1910b, p. 656).
 Epidermal cell secretion, Saguchi (1915, p. 395).
 Epidermal fibrils, Firket (1911, p. 537).
 Fat (neutral), Altmann (1889, p. 94).
 Fibrils of Herxheimer, Favre and Regaud (1910, p. 1138).
 Filament hélicoïdal, Perroncio (1910, p. 310).
 Fucasin, Le Touze (1912, p. 33).
 Glycogen, Arnold (1908, p. 365); Alexicief (1916a; 1916b, and 1917a).
 Goblet cell mucus, Grynfeltt (1913, p. 11).
 Grains, chromophiles, Leplat (1913, p. 219).
 Grains safranophiles, Regaud (1910, p. 342).
 Granules de ségrégation, Renaut and Dubreuil (1906b, p. 230).
 Hemoglobin pigment, Ciaccio (1911, p. 16).
 Hemoglobin crystals, Policard (1912a, p. 92; 1912b, p. 230).
 Leucites (= leucoplasts).
 Leucoplasts, Lewitsky (1910, p. 542).
 Lipoid in nerve-cells, Cowdry (1914a, p. 13).
 Liposomes, Fauré-Fremiet (1910b, p. 538).
 Macromitosome, Gatenby (1917, p. 416).
 Mammary-gland secretion, Hoven (1911, p. 325).
 Melanin, Asvadourova (1913, p. 263).
 Metachromatic corpuscles, Guillaiermond (1913d, p. 439).
 Mucorine, Moreau (1915a, p. 171).
 Mucus (boules pieriphiles), Grynfeltt (1913, p. 11).
 Myofibrils, Benda (1899a, p. 379).
 Nebenkern in spermatogenesis, Meves (1900, p. 585).
 Neurofibrils, Meves (1907a, p. 403).
 Neuroglia fibrils, Meves (1907a, p. 403).
 Pancreatic zymogen, Hoven (1910b, p. 349).
 Parathyroid secretion, Bobeau (1911, p. 186).
 Parotid secretion, Regaud and Mawas (1909b, p. 220).
 Pheoplasts, Nicolosi-Roncati (1912b, p. 144).
 Pigment, Prenant (1913, p. 926).
 Poison-gland secretion, Torraca (1916, p. 326).
 Pole-disc granules in chrysolimid eggs, Hegner (1914, p. 419).
 Prepuccial-gland secretion, Altmann (1890, p. 99).
 Prostate secretion, Akatsu (1903, p. 566).
 Randeifien of blood corpuscles, Meves (1905, p. 103).
 Retinal pigment, Luna (1913b, p. 56).
 Russell's bodies, Dubreuil and Favre (1914a, p. 373).
 Sebaceous-gland secretion, Nicolas, Regaud and Favre (1912a, p. 203).
 Sheath of tail-thread of sperm, Meves (1900, p. 586).
 Spiral filament of sperm, Benda (1897, p. 412).
 Starch, Guillaiermond (1912a, p. 412).
 Submaxillary-gland secretion, Regaud and Mawas (1909b, p. 220).
 Suprarenal pigment, Mulon (1913, p. 1026).
 Suprarenal secretion, Mulon (1910b, p. 873).
 Sweat-gland secretion, Nicolas, Regaud and Favre (1912b, p. 191).
 Tannin, Guillaiermond, (1913d, p. 437).
 Test of foraminifera, Fauré-Fremiet (1911, p. 120).
 Trophoplasts (= leucoplasts).
 Thyroid-gland secretion, Grynfeltt (1912c, p. 147).
 Tyrosin, Asvadourova (1913, p. 263).
 Tonofibrils, Saguchi (1913, p. 239).
 Urea in the kidney, Oliver (1916, p. 318).
 Venom, Grynfeltt (1913, p. 11).
 Vitellus, Loyez (1909, p. 191).
 Xanthophyllous pigment, Guillaiermond (1913h, p. 1926).
 Yolk, Coghill (1915, p. 349).

The term "transformation" has been used too freely and too loosely. Investigators speak glibly of the transformation of mitochondria into other materials without stopping to think what it means. We can understand the transformation of a liquid into a gas, but we can not conceive of the transformation of oxygen into carbon. The likelihood of a transformation taking place depends upon the difference in the properties of the original and the transformed substances; but the differences in the properties of mitochondria and all these materials are usually ignored. Accordingly, some of the statements involve chemical and physical impossibilities. We have good reason to suppose that mitochondria resemble phospholipins, and it is therefore incredible that they should transform into hemoglobin which contains iron, chlorophyll which contains magnesium, and the colloid of the thyroid gland with its iodine. The iron, magnesium, and iodine can come only from the cytoplasm. The question, however, is vastly complicated by statements, apparently supported by fairly good evidence, to the effect that mitochondria consist,

in addition to the phospholipin, of an albuminous substratum of some sort. Nevertheless, in the vast majority of cases a true transformation is out of the question.

The idea of a transformation is often based upon the mere observation of substances within the mitochondria. The fallacy of this line of reasoning is evident, for no one would say that because the cell contains iron or phosphorus the iron or phosphorus is produced by a transformation of the substance of the cell. Substances unquestionably penetrate into the mitochondria from the cytoplasm, as shown by the fact that in these supposed transformations of mitochondrial material into something else there is always a distinct increase in size. For instance, the mitochondria possessing bleb-like swellings in gland-cells are larger than those without them, and mitochondria containing starch, fat, pigment, crystalloids, and other materials are invariably greatly enlarged. In cases where granular mitochondria expand to form vesicles careful observation will often show that there has been little or no change in the absolute amount of mitochondrial substance; it has simply become spread over a larger area. We may safely regard this imbibition from the cytoplasm as established, but how the materials are taken in is exceedingly difficult to explain. It differs sharply, however, from the normal process of growth because the expansion is lateral, while in growth extension is usually longitudinal (p. 70). I incline strongly toward Regaud's ectosome theory (1909*a*, p. 919), according to which mitochondria play the part of plasts choosing and selecting substances from the surrounding cytoplasm, condensing them and transforming them in their interior into infinitely diverse products; but I would venture to emphasize the fact that in all this the mitochondria may be acting in an entirely passive manner as a vehicle, taking up materials by virtue of their phospholipin constitution, or on account of physical forces acting on their surfaces, or for other reasons, and that the optically homogeneous ground-substance of the cytoplasm may be the active and essential agent in this as in so many other vital manifestations. No change of the mitochondrial substance need be involved. Sugar, which is heaped up in plasts, is certainly not formed through a transformation of mitochondrial material, or of the plasts which contain it. They merely act as containers, the foreign material being localized in certain regions of the filament.

But in rare cases there is evidence of an actual change in the mitochondria themselves, especially in the formation of fibrillar structures, in cornification, and in other similar processes. We may conceive of this as taking place in several ways: (1) by the addition of substances from the cytoplasm which enter into close combination and become integral constituents of the mitochondria; (2) by the mitochondria giving up to the cytoplasm certain of their normal constituents; (3) by chemical dissociation which may or may not be followed by resyntheses. Fat, lipid, and other similar substances might be formed; but it is important to bear always in mind that the possibility of a transformation diminishes in direct proportion to the degree of dissimilarity between the mitochondria and the material in question. It is for this reason that I am willing to entertain almost any alternative hypothesis rather than accept unqualified statements of the chemical transformation of mitochondria into dissimilar substances.

FIBRILS.

COLLAGENIC FIBRILS.

Meves's own work (1910*a*, p. 164) on the histogenesis of collagenic fibrils may now be briefly mentioned. His material consists of a series of preparations of the growing tendon in the posterior extremity of chick embryos of from 6 to 19 days' incubation. He fixed the embryos in a modification of Flemming's fluid, cut some of the specimens in transverse, others in longitudinal sections, stained them with iron hematoxylin, and counterstained with acid fuchsin. In this way the mitochondria were colored black and the fibrils red.

The illustrations of the preparations on which he bases his contention are beautifully shown in his second plate (Tafel III). They are arranged in two series of increasing grades of differentiation, the uppermost of which is taken from longitudinal sections and the lower from transverse sections. They show that the mitochondria become accumulated in the peripheral parts of the cytoplasm in stages during which the collagenic fibrils first appear. The mitochondria are filamentous, but are not so long as the primitive fibrils, the ends of which he was unable to observe. The figures show, in addition, that there is a decrease in the number of mitochondria in the cells of later stages and that the mitochondria are no longer most abundant in the peripheral parts of the cell. Meves's line of reasoning is instructive. He says (p. 164):

"Was nun die Deutung der beschriebenen Bilder anlangt, so bleibt meiner Meinung nach nichts anderes übrig, als anzunehmen, dass die Bindegewebsfibrillen aus Chondriocenten hervorgehen. Die Chondriocenten werden zunächst epicellulär. Sie ändern dann ihre chemische Beschaffenheit, indem sich ihre Substanz in eine solche umwandelt, welche weder durch Eisenhämetoxylin noch durch Fuchsin färbbar ist. Auf diesem Stadium treten diejenigen von ihnen, welche in einer Reihe liegen, untereinander mit ihren Enden in Verbindung. An der Bildung einer Fibrille beteiligen sich zahlreiche Zellen (alle diejenigen, denen ihr Verlauf fest anliegt), indem jede einen Fibrillenabschnitt liefert. Die Fibrillen ändern dann zum zweiten Mal ihre chemische Beschaffenheit, indem sie eine intensive Färbbarkeit für die Collagenfarbstoffe gewinnen. Schliesslich werden sie von den Zellen frei und kommen in den Spalträumen zwischen ihnen zu liegen."

Before we enter upon a criticism of Meves's work it is necessary for us to recognize, in all fairness, that he does not claim to have conclusively established the transformation of mitochondria into collagenic fibrils, since, as he himself emphasizes, the steps which lead up to this conclusion consist of assumptions as well as of positive evidence. It is important above all to note that, according to Meves, the mitochondria (chondriocentes) are invisible, not staining with either iron hematoxylin or fuchsin when they form the fibrils. This assumption that they are invisible when the most important stage of the whole process is taking place disarms all criticism at the outset.

Meves makes his chain of evidence ("Kette der Beweise," p. 165) still more fragile by asking the question: "If the chondriocentes have nothing to do with the formation of fibrils, why then do they become epicellular?" I do not know whether Meves means by the term "epicellular" that the mitochondria are actually out-

side the cells, lying upon them, or that they are simply accumulated in the peripheral parts of the cytoplasm within the cell-membrane. The derivation of the term and the appearance of certain mitochondria illustrated in his figure 25 seem to support the first interpretation. The difficulty of asserting that mitochondria in Meves's preparations are without the cell-wall is great, not only because the cell-walls are not differentially stained, but also by reason of the mode of deposition of collagen. It seems, therefore, that what Meves describes is simply a heaping-up of mitochondria in the cytoplasm beneath the cell-membrane.

A similar peripheral arrangement of mitochondria in the cytoplasm has been described in a whole host of conditions other than fibril formation and therefore is without special significance in this connection (see p. 76).

The filamentous shape of the mitochondria might at first sight appear to be indicative of a transformation into collagenic fibrils. This is, however, not the case, because mitochondria, which are also thread-like, occur in the same stage of development in cells which do not form fibrils.

Meves's other argument that the mitochondria decrease in number as the fibrils form in the course of development would be valid only could it be shown that the diminution in number was not brought about in some other way. Indeed, it is only one instance of a very general phenomenon, that the mitochondria grow fewer and fewer in all cells as they grow older. Meves, himself (1911*a*, p. 495), has shown this to be the case in the later stages of the cytomorphosis of red blood-cells of the guinea-pig; Firket (1911, p. 544) has demonstrated the same phenomenon in the cells of the egg tooth of chick embryos; and Regaud and Favre (1912, p. 328) have confirmed his results by their observations on the epidermis of man.

Meves's hypothesis of the rôle of mitochondria in connective-tissue fibril formation can not, apparently, be reconciled with Baitsell's (1916, p. 754) recent work on wound healing. Baitsell discovered that certain fibrils form, quite apart from the cells, as a differentiation of a typical fibrin net in the coagulation tissue between the cut surfaces. The cells all wander in later. The staining reactions of the new fibrous tissue, formed in this way, appear to resemble in many ways those of true connective tissue. Here we are dealing with the formation of fibrils like those of connective tissue from a known substance, fibrin, in the absence of cells, and, since the mitochondria are always intracellular, it is inconceivable how they could be bodily transformed into the fibrils, as Meves claims.

It is interesting also to note that M. R. Lewis (1917, p. 56) has made a careful study of the behavior of mitochondria and fibrils in cultures of subcutaneous tissue of chick embryos. The fibers in the explanted piece were not observed to grow either in length or bulk, but new fibrils arose as delicate lines in the exoplasm of the cells and quite independently of the mitochondria. The cessation of growth in the one and the initiation in the other may indicate some superficial, or perhaps fundamental, difference. No attempt was made to compare the microchemical reactions of the new-formed fibrils with the definitive connective-tissue fibrils in the organism. The observations indicate the independence of mitochondria and afford a plausible alternative hypothesis of the development of connective-tissue fibrils.

It may even be said that Meves's observations, instead of proving that the mitochondria are transformed into collagenic fibrils, indicate that the two are quite distinct: for if there is, as he assumes, a change in the chemical constitution of mitochondria so that they do not stain with either iron hematoxylin or fuchsin, one would expect to see some evidence of this in his figures. But his figures show that the mitochondria are characterized by the extreme uniformity of their reactions to iron hematoxylin; they show no variability whatever. Again, if, as he further assumes, there is another change in the chemical constitution of the invisible fibrils (invisible by his own assumption) by which they acquire an intense affinity for collagen-staining dyes, one would look for some variation in the staining with the said collagen dyes. But the figures show that there is no variation in the reactions of the fibrils. The third point, which may be justly urged, is his last assumption that in later stages the fibrils are differentiated by virtue of a formative activity. It follows that the theory of the origin of the fibrils from mitochondria is applicable only to a very limited stage in their formation and does not fit the facts which he himself has observed relative to their formation in older embryos.

The argument from analogy advanced by Duesberg (1912, p. 759), that the formal proof of the rôle of mitochondria in the formation of myofibrils may be regarded as indirect evidence of their participation in the development of other formed elements (collagenic fibrils and neurofibrils) cuts both ways; for it is equally true that the notable absence of evidence in favor of the formation of collagenic fibrils by a transformation of mitochondria leads one to doubt a like origin of myofibrils.

MYOFIBRILS.

Although Benda (1899*a*, p. 379) and Meves (1907*a*, p. 402) were the first investigators to claim that mitochondria became changed into myofibrils, Duesberg (1909, p. 126, and 1910, p. 647) has furnished the most complete evidence in support of this contention. His material consists of a very complete series of chick embryos of from 19 hours' to 10 days' incubation prepared by Benda's method for mitochondria. He employed also, for control, sublimate acetic, alcohol, and other fixatives, and the chloride-of-gold method of Ranvier. He studied both the myotomes and the myocardium, but his most detailed work related to the former. He found that at first the mesoblastic cells contain only typical mitochondria in the form of granules, rods, and short, wavy filaments, and that in more advanced stages of development filaments of the same girth and morphological characters became more and more numerous and of greater and greater length, until they began to show traces of differentiation into segments. The mitochondria and the homogeneous filaments stained alike by the Benda method, but after the first indications of segmentation appeared the staining reaction of the fibril began to change, for they no longer stained as deeply as the mitochondria. His figures indicate a marked decrease in the amount of mitochondria parallel with the differentiation of fibrils. This is seen by a comparison of figure 14 with the succeeding ones, figures 16, 20, 23, 25, and 26. He arrived at the conclusion that the mitochondria elongate and become transformed into myofibrils.

Von Kurkiewicz arrived at the same conclusion concerning the mitochondrial origin of the fibrils in the heart-muscle of the chick. Schultze also claims to have confirmed Duesberg's contention regarding the rôle of mitochondria in myofibril formation. Brück (1914, p. 581) has described the mitochondrial origin of myofibrils in *Anodonta cellensis*. Moreover, Leplat (1912, pp. 458 and 509) has studied the development of fibrils in *Mm. sphincter pupillæ* and *ciliaris* of birds by the application of the Benda method. He was able to observe all the stages in the differentiation of the myofibrils which Duesberg described and he reaches the same conclusion. The following additional investigators favor the doctrine of the transformation of mitochondria into myofibrils: Lewitsky (1910, p. 539), Favre and Regaud (1910, p. 1138), Hoven (1910*a*, p. 476), Prenant (1911*a*, p. 463), G. Arnold (1912*a*, p. 289), Schäfer (1912, p. 193), Luna (1913*c*, p. 478), Jordan and Ferguson (1916, p. 94), and others. Heidenhain (1911, p. 1086), Levi (1911, p. 191), and Gurwitsch (1913, p. 123) are in the minority in that they do not subscribe to it.

The accuracy of the facts forwarded by Duesberg being beyond cavil, it becomes necessary for us to determine whether they permit of any other interpretation except that advanced by him. The nature of the unsegmented fibrils and the significance of the fluctuations in the amount of mitochondria are important points. Apparently the technique employed is not specific, for it colors the mitochondria and the primitive fibrils in the same way, although they differ in their microchemical properties, because we find that the mitochondria are dissolved by fixatives containing a concentration of acetic acid which in no way affects the myofibrils. They also react differently to stains. If, therefore, differences of this nature are not revealed by the technique employed, the possibility must be entertained that the elongated homogeneous filaments described by Duesberg may differ *inter se*; in other words, that we may be dealing with two different kinds of filaments which may appear similar on account of the stain which is used, one of which is mitochondrial, the other a precursor of the definitive myofibrils which does not possess the properties of mitochondria; so that Duesberg's investigations do not exclude the possibility of the origin of myofibrils from material which is not mitochondrial. In fact, there is some indication of the existence of non-mitochondrial precursors. I refer, for instance, to the observations of Godlewski (1902, p. 149) and others, according to which the myofibrils result from the confluence of small masses of material, not through the elongation and transformation of a homogeneous filament.

Moreover, I have observed in my own preparations of chick embryos of 100 hours' incubation (stained with fuchsin and methyl green) very delicate green-colored fibrils, side by side with others stained red, and still others beginning to show traces of segmentation. I have also seen similar fibrils stained red with alizarin in Benda preparations and light gray with iron hematoxylin. Morphologically they resemble the filamentous mitochondria, but their staining reactions are entirely different. Whether they give rise to the definitive fibrils or not I can not say.

Duesberg (1915, p. 59) has supplemented his discovery of the abundance of the mitochondria in the myoblasts and their subsequent diminution during fibril formation by important investigations on ascidians, where he found that the mitochon-

dria are particularly numerous in the primordial muscle-cells, often being arranged in chains, indicative, he thinks, of transformation into fibrils. Moreover, Romeis (1913*a*, p. 10) has found a marked increase in mitochondria and transition forms like those figured by Duesberg during the regeneration of muscle-cells in *Triton*.

Too much weight should not be placed in the presence-and-absence argument. There may be other reasons than fibril formation for oscillations in the amount of mitochondria. Romeis attributes the increase in mitochondria to the more embryonic condition of the cell. It is very possible that it may depend upon increase in oxidations (see p. 82). Even should there be some association between the decrease in the number of mitochondria and the formation of fibrils, it does not follow that the mitochondria themselves change into them. The arrangement of mitochondria in chains may simply be the outward and visible sign of the formation of fibrils between them from non-mitochondrial precursors.

The results thus far obtained with tissue cultures by Levi (1916*c*, p. 82) are difficult to reconcile with Duesberg's view, for Levi found that there was no relationship whatever between mitochondria and the growth of fibrils in mesenchyme cells.

We hold, not without some justification, that mitochondria are chemically a combination of phospholipin with a small fraction of albumin. Now we are asked to believe that, at a certain stage in the development of the embryo, filamentous mitochondria, which to all our solubility tests and staining reactions are alike and show no variability, in three different localities become chemically transformed into three different materials. In the myoblasts they are said to change into myofibrils, which contain tyrosin; in the neuroblasts they are supposed to change into neurofibrils, the chemical nature of which is unknown; and lastly, they are also said to form connective-tissue fibrils, which yield collagen, a protein devoid of tyrosin. But the mitochondria do not contain tyrosin (Cowdry, 1916*a*, p. 427). Where, then, does it come from? Certainly not from the mitochondria. Other more difficult questions must be asked and answered before we can bring ourselves to believe in the chemical transformation of mitochondria into myofibrils.

EPIDERMAL FIBRILS.

Firket (1911, p. 537) has investigated the rôle of mitochondria in the differentiation of epidermal fibrils in the egg tooth and feathers of chick embryos. His material consists of three series of chick embryos. The first, from 8 to 15 days' incubation, was fixed in Bouin's fluid; the second, from 6 to 21 days, in Flemming's fluid as modified by Meves; and the third, embryos from 6½ days until hatching, in Benda's fluid. Preparations from the first were stained in safranin or iron hematoxylin, either alone or followed by a counterstain of rubin, eosin, and orange G. Some sections from the second and third series were also treated in this fashion, although the majority were stained with iron hematoxylin or crystal violet.

Firket found (p. 540) a certain variability in the coloration of the fibrils after fixing in Bouin's fluid and staining with iron hematoxylin. The first ones to appear were lighter colored and stained irregularly, whereas the completely differentiated fibrils stained a darker uniform shade. He says (p. 544) that this "mon-

liforme" appearance leads one to suppose that the transformation into epidermal fibrils takes place in several places in the substance of a single chondriokont. According to him, the fibrils are at first basophile, become acidophile, and are finally masked, the whole cell assuming a homogeneous aspect. In the preparations which he fixed in Meves's fluid and stained with either iron hematoxylin or krystallviolett, both the mitochondria and the fibrils were stained. He writes (p. 542) that as one examines the cells of more and more superficial layers of the corps muqueux inférieur, one sees part of the chondriosomes elongate and assume an undulating appearance; in the same cells other chondriosomes retain their primary dimensions. The latter become less and less numerous as one approaches the corps muqueux inférieur. Soon almost all the chondriosomes have assumed the form of long undulating filaments, which he says undoubtedly constitute the first-formed epidermal fibrils. Since he found that the number of completely formed fibrils in a cell greatly exceeds the original amount of mitochondria, he concludes that the fibrils formed by the transformation of chondriosomes multiply by longitudinal division, although neither he nor Branca observed it. He bases this conception on two considerations: (1) that isolated epidermal fibrils are generally of finer diameter than the chondriokonts; (2) that the division of other fibrillar formations such as myofibrils and neurofibrils is admitted by most authors.

Duesberg's observations on the epidermis of the tadpole lead him to the same conclusion that the epidermal fibrils arise by the transformation of mitochondria (1912, p. 796).

Since the iron-hematoxylin method and the Benda method color the mitochondria and the completely formed fibrils (two very different structures microchemically) alike, these methods of technique can not be regarded as suitable for an investigation of this nature. More specific methods must of necessity be employed. It follows that Firket, in his series showing a parallelism between the disappearance of mitochondria and the formation of fibrils, has had to rely solely upon the diameter of the filaments to determine whether they are mitochondria or differentiated fibrils. His position is therefore insecure, since he can not distinguish with certainty the structures between which he claims to show transitions. If we admit that this parallelism does exist, we find that it is capable of a similar explanation to that advanced in the discussion of collagenic fibrils and myofibrils, namely, that the amount of mitochondria is diminished because the activity of the cells is lessened in the later stages of cytomorphosis.

The problem is rendered more difficult and deceiving because of the superficial resemblance which obtains between the staining reactions of the mitochondria and of the fibrils. Some of the fundamental differences which are said to exist between them may be indicated:

MITOCHONDRIA.

1. Granules, rods, filaments.
2. Soluble in fluids containing an excess of acetic acid, *e. g.*, Zenker's fluid
3. Destroyed by fixation in Bouin's fluid (Firket).
4. Acidophile in pancreas after fixation in non-mordanting fluids (Bensley, 1911, p. 362).

FIBRILS.

- Long threads of finer diameter (Firket, p. 544).
Resistant to acetic acid in fixatives.
- Well preserved by Bouin's fluid (Firket).
First basophile, becoming later acidophile (Firket, p. 539).

Firket's evidence is not conclusive, because he has not demonstrated the existence of a complete series of transitional stages showing the loss in the properties of the fibrils. He has, however, gone further than Meves did in connection with the collagenic fibrils, or Duesberg in the case of the myofibrils, because he succeeded in demonstrating a variability in the staining reaction of the first fibrils to appear.

NEUROFIBRILS.

Many investigators have touched on the question whether mitochondria play a part in the differentiation of neurofibrils, but Hoven (1910*a*, p. 427) in particular, working with chick embryos, has furnished the most complete evidence in favor of the view that mitochondria are actually transformed into them.

This interesting conception has been supported by Meves, who originally enunciated it (1907*a*, p. 403), as well as by G. Arnold (1912*a*, p. 288), and several others to be mentioned subsequently; it has been rejected by Marcora (1911, p. 952), Levi (1911, p. 180), and Gurewitsch (1913, p. 126), while Duesberg (1912, p. 745) has assumed a non-committal attitude with regard to it.

It is based upon the following statements:

(*a*) That the neurofibrils increase in amount as the mitochondria decrease, until finally the adult condition is attained in which the neurofibrils are completely differentiated and the mitochondria absent (Hoven, 1910*a*, p. 478; Meves, 1910*b*, p. 655).

(*b*) That microchemical transitions exist between mitochondria and fibrils, since the primitive neurofibrils may first be stained by mitochondrial methods, then by both mitochondrial and neurofibrillar methods, and finally by the various neurofibrillar methods of technique alone (Meves, 1908, p. 838; Hoven, 1910*a*, p. 478, etc.).

(*c*) That morphological transitions also exist between mitochondria and neurofibrils; according to Meves (1908, p. 838), chains of mitochondria are changed into neurofibrils; according to Hoven (1910*a*, p. 475), the mitochondria form a reticulum from which the neurofibrils are differentiated.

(*d*) That the development of myofibrils, connective-tissue fibrils, and the fibrils in epithelial cells support this theory, since they, in a similar fashion, are developed from mitochondria. This constitutes the argument from analogy (Meves, 1907*a*, p. 403; Duesberg, 1910, p. 613; Meves, 1910*a*, p. 162; Firket, 1911, p. 545; and Duesberg, 1912, p. 759).

The bearing of my own observations (1914*d*) upon the statements upon which the theory of the mitochondrial origin of the neurofibrils rests is as follows:

(*a*) My own findings are utterly at variance with the first argument, for I can discover no decrease in the amount of mitochondria running parallel to the formation of neurofibrils. Moreover, the statement that they are absent in the adult condition is wholly unwarranted in view of the fact that several investigators had already unquestionably seen mitochondria in adult nerve-cells (p.101).

(*b*) The second statement postulates the existence of three distinct phases in the development of the neurofibril, each of which is characterized by certain microchemical properties. In the first stage the primitive neurofibrils may, it is

said, be stained by mitochondrial methods; in the second by both mitochondrial and neurofibrillar methods; in the third by the various neurofibrillar methods of technique alone. I have found (and I have already described the fact) that structures which we are accustomed to call neurofibrils may in truth be stained by certain mitochondrial methods. I refer to the iron-hematoxylin method of Meves, the Benda method, and the anilin fuchsin methylene-blue erythrosinate and toluidin-blue methods of Bensley, but the staining is not specific and depends on the degree of differentiation. A comparison of figures 21, 26, and 25 published in my 1914*d* paper will be sufficient to show that this is true in the case of the last-mentioned method. These three figures have been drawn from neighboring sections of the same embryo of 100 hours' incubation, mounted on the same slide, and stained with anilin fuchsin and toluidin blue. In the first figure, the differentiation is practically *nil*, the mitochondria staining exactly the same color as the neurofibrils; in the second figure it has been carried a little further, with the result that the neurofibrils have lost their bright crimson color and have assumed a dull red shade; while in the last (fig. 25) the decolorization has been carried to an extreme, so that the neurofibrils have lost all of the acid fuchsin and have become stained with the differentiator, toluidin blue. It is to be noted that in these progressive stages of differentiation the initial affinity of the neurofibrils for the acid dye (acid fuchsin), in which they resembled mitochondria, is gradually changed to an affinity for a basic dye (toluidin blue), while the intensity of the coloration of the mitochondria with the acid fuchsin remains unaltered. Furthermore, the fact that the coloration of the neurofibrils by mitochondrial dyes is marked in adult cells, which I have mentioned in a preceding contribution, should be taken into consideration before regarding it as indicative of the existence of transitions between mitochondria and primitive neurofibrils.

Let us now consider the statement that the primitive neurofibrils may be stained by both the mitochondrial and the neurofibrillar methods (*i. e.*, the second phase). The completeness of the demonstration of mitochondria by the iron-hematoxylin method depends upon the presence in the fixative of chromic acid, osmic acid, and acetic acid, in suitable amounts, and on the mordanting action of iron alum, while their complete absence in the neurofibrillar preparations is due to the unmodified action of silver nitrate. The neurofibrils seem to have a special affinity for silver nitrate, upon which all silver impregnation methods depend. So it is extremely unlikely, especially in the absence of direct evidence, that so widely divergent methods stain the same thing, namely, the primitive neurofibrils. Of course it will be argued by the adherents of this theory that the Italian investigators have succeeded in demonstrating mitochondria by modified Golgi methods, but there is a long step between this fact and proving that the mitochondria in a certain specified stage in the developing nerve-cell may be stained interchangeably by mitochondrial and neurofibrillar methods. If this should be the case in other stages when neurofibrils are not being formed, and in other tissues, it would of necessity be deprived of the significance which investigators have been inclined to attach to it.

Finally, the neurofibrils are said to enter upon a third phase in their history characterized by the loss of their affinities for mitochondrial dyes. I have nevertheless failed to find any conclusive evidence that the neurofibrils change their chemical properties after their first formation. My failure may be due to the unstandardized condition of the neurofibrillar methods of technique which still prevails. In any case the burden of supplying the evidence rests with those who make the statement. If the neurofibrils are formed by a chemical transformation of mitochondrial substance into neurofibrillar material, one would expect to find variations in the effects of fixation and in the staining properties of mitochondria during their formation. I have shown that the exact converse obtains. Both the solubility of mitochondria in acetic acid and the staining reactions of mitochondria in the cells of the neural tube in which neurofibrils are being actively formed remain remarkably uniform and constant. Moreover, these properties apparently differ in no wise from those of mitochondria in the neural tube before the formation of neurofibrils or from the mitochondria in other embryonic cells. It is evident, therefore, that the facts do not justify the statement that microchemical transitions exist between mitochondria and neurofibrils.

(c) With respect to the evidence for morphological transitions I would state that I have failed to confirm Meves's contention that chains of mitochondria are transformed into neurofibrils. Mitochondria are sometimes oriented end to end, and one may often observe very long filamentous forms. It is a very far cry from a linear arrangement of mitochondria or from long filamentous mitochondria to neurofibrils. This is manifested, among other things, by the fact already mentioned, that there is nothing peculiarly distinctive about the morphology or the arrangement of mitochondria in the cells of the neural tube during neurofibrillar formation; they are alike indistinguishable, on the basis of their morphology and distribution, from the mitochondria in the cells of the neural tube in stages prior to the differentiation of neurofibrils, and from the mitochondria occurring in other embryonic tissues both before, contemporaneous with, and after the development of neurofibrils. Therefore, on the ground of the shape and cytoplasmic arrangement of mitochondria, there is just as much evidence for the formation of neurofibrils in structures derived from mesoderm and endoderm as there is in the case of the neural tube.

(d) The value of the argument from analogy has already been made plain by the preceding discussion of the development of other fibrillar structures.

It seems clear that the neurofibrils are not formed by a transformation of mitochondria. They are elusive structures. They can not be seen in the living cell, or with the aid of vital dyes; neither can they be dissected out (Kite¹). There is every reason to believe that they do not occur in the living cell in the form in which we see them in our silver preparations. They are formed of material quite different from the Nissl substance, mitochondria, or canalicular apparatus, though we neither know what it is nor the factors concerned.

¹Dr. Kite, personal communication.

It has been claimed that many other fibrillar structures are formed from mitochondria. Saguchi (1913, p. 239), for example, has made a careful study of the development of the *tonofibrils* and *intracellular structures of Eberth* in the epidermal cells of batrachian larvæ and concludes that they arise through a chemical transformation of mitochondria. His figures show a very definite association of the mitochondria with the fibrils and all morphological gradations between the two are represented. His evidence, however, for a chemical change (p. 241) is not conclusive, for it does not exclude the possibility that the substances making up the fibrils arise elsewhere and are deposited within the mitochondrial filaments without change of the mitochondrial material. The *fibrils of Herzheimer* are considered by Favre and Regaud (1910, p. 1138) to be true mitochondria. Meves (1907a, p. 403) has suggested that the *neuroglia fibrils* are likewise developed from mitochondria, but has forwarded no evidence in substantiation.

In concluding this discussion of the histogenesis of fibrils it may be remarked that it is somewhat illogical to suppose that substances of such diverse chemical constitution are all formed through the transformation of a single substance, a phospholipin, combined perhaps with a small fraction of albumin. The collagenic fibrils on boiling yield gelatin, a protein devoid of tyrosin; the neurofibrils are of unknown composition, and we even doubt their existence in the living cell; certain of the epidermal fibrils contain a keratin-like material, and our chemical acquaintance with the neuroglia fibrils is of the slightest. It seems far more likely that the fibrils are, from the outset, different from one another—that they are formed from different materials rather than from the same material. They may be formed through a condensation of substances in the cytoplasm, either in the form of minute, perhaps ultramicroscopic, particles which tend to be arranged in rows following lines of stress or strain in the cell and which naturally fuse together, end to end, in accordance with the law of least surfaces; or, it is entirely conceivable that there may be only a single center of condensation which grows and enlarges by the addition of more material by accretion, something like a crystal. In connection with the first of these alternatives we know that lines of stress do exist in living protoplasm, for the fibrils are deposited at a time in development when growth activities are greatly pronounced, when cellular migrations are common, and changes in the form of various parts of the body quite frequent. The fully formed fibrils undoubtedly correspond, in position, to lines of stress and strain. We know that certain cytoplasmic elements are subject to orientation along such lines. I refer, particularly, to the arrangement of material in lines about the centrosomes, to the arrangement of granules in the rootlets of cilia, to the characteristic deposition of material in bone, etc.

PLANT PLASTIDS.

By far the most convincing evidence in favor of a participation of mitochondria in histogenesis, through an actual chemical transformation of their substance, is to be found in the botanical literature. In fact, these newer methods of mitochondrial technique strike at very important problems, for they have a definite bearing upon the origin of all plastids.

Lewitsky (1910, p. 542) and Pensa (1910, p. 325) deserve credit for opening up this important field almost simultaneously. Lewitsky studied mitochondria in growing asparagus tips. He claims to have discovered that the leucoplasts arise from them, and advances the general conclusion that the chondriosomes (mitochondria) in plant cells are to be regarded as formative granules, just as they are supposed to be in animal tissues. In other words, he extends Meves's important generalization (p. 101) to plant tissues. Pensa arrived at essentially similar results, and the problem has since been flooded with contributions from all quarters.

We have to consider the formation of leucoplasts and starch; of chloroplasts and chlorophyll; of chromoplasts and pigments of almost infinite variety; and of the elaioplasts, which elaborate fats. Let us analyze the facts observed and see whether they permit of any other interpretation than that of direct chemical transformation. It can not be denied that there is a very definite topographical relation between the mitochondria and the deposition of these substances, for they are actually laid down within them, but it is too much to say that we are here dealing with an actual chemical transformation of the mitochondrial substance. Yet this is just the claim that Guilliermond (1912*a*, p. 394) makes. He has shown that filaments which possess these swellings are chemically different from the other mitochondria in the cell, because their solubilities in certain fixatives are different; but it does not follow, nor is there any reason to suppose, that further alterations occur in the constitution of the mitochondria by which they become changed into starch, pigments, chlorophyll, and so on. Guilliermond (1912*a*, p. 408) points out at length that Hoppe Seyler and others look upon chlorophyll as being a combination of lecithin and other substances, and that it accordingly resembles mitochondria quite closely. The other pigments and the crystalline substance "carotene" differ widely from mitochondria chemically. To my mind, the facts observed, which no one would question, demonstrate nothing more than that the said topographical relationship between mitochondria and the formation of these materials exists. These substances are simply deposited, or accumulated, or heaped up within the mitochondria, which serve as a convenient and suitable vehicle, by virtue of their chemical and physical properties. There is good reason to believe that some of the substances, like the pigments, may be soluble within them. They may also act as condensers, as Regaud believes. But what I want to make absolutely clear is that this does not necessarily involve any chemical change whatever in their constitution.

The fact that mitochondria diminish in number, *pari passu*, with differentiation in plant cells as well as in animal cells, does not mean that they change chemically into the products of differentiation, as some investigators have tacitly assumed, for this decrease in amount of mitochondria is probably associated with a decrease in the rate of metabolism which we know occurs with differentiation and senility. The evidence now at hand that the mitochondria are concerned with metabolism is given in detail on page 131. There is nothing to indicate that the mitochondrial substance about the granule of starch, or the pigment, as the case may be, is chemically transformed into something else as it decreases in amount. It may simply

become resorbed and go into solution in the cytoplasm, for we have good reason to believe that the mitochondria do go back into solution (p. 98). These are not all the points that must be cleared up before we can look upon this question of the actual chemical transformation of mitochondria into plastids as being definitely settled. The work which has been done so far is extremely suggestive, but it is not conclusive.

The evidence seems to point to the conclusion that the single chloroplasts in some of the algae do not arise from mitochondria, because mitochondria are absent, so that they would constitute an important exception to the general rule. It is thought, moreover, that these single plastids perform a similar function to that of those in the adult cells of higher plants, where the mitochondria are either absent or greatly reduced in number. This, by Sapehin, is brought forward as evidence against the view that mitochondria are transformed into plastids. It is necessary to find out whether the chloroplasts in animals, some varieties of *Paramoecium* for example, are formed from mitochondria in the same way that has been claimed in plants. Information on this point is urgently needed, because it would tell us whether animal mitochondria are capable of performing the same feats which have been ascribed to vegetable mitochondria, and give us an idea of the degree of resemblance of mitochondria in the plant and animal kingdoms. Varying degrees of chlorophyll production, caused by regulating the illumination, should be studied. It would be interesting to note whether phosphatids outside the body are capable of entering into close combination with starch and chlorophyll. And, finally, experiments might be devised to show whether animal mitochondria and plant mitochondria, in species devoid of chlorophyll, are able to pick up and condense starch and chlorophyll when they are brought into intimate contact with them, which would have a very important bearing upon this question of the transformation of mitochondria into plastids.

PIGMENTS.

HEMOGLOBIN.

Ciaccio (1911, p. 16), in October, arrived at the conclusion that hemoglobin is formed under the influence of mitochondria on the basis of his observation that, in the rabbit, not only the basophilic erythroblasts but also those provided with hemoglobin, and the erythrocytes, just before they enter into the circulation, contain typical mitochondria. In October, also, Meves (1911*a*, p. 495) published identical results relating to the red blood-cells in the bone marrow of the guinea-pig.

A few months later, Schridde (1912, p. 516) attempted to go further than Ciaccio and Meves by claiming that the mitochondria diminish in number in direct proportion to the increase in the hemoglobin, and, on the basis of this, concluding that the plastosomes (mitochondria) are the formers of the hemoglobin.

This was followed almost immediately by a second paper by Ciaccio (1913*b*, p. 393), in which he says that there is no foundation for Schridde's opinion. He holds to his original view that the part played by the mitochondria is quite indirect. His chief and only objection appears to be that the acid fuchsin, which Schridde

employed, colors the hemoglobin intensely and may mask or hide the mitochondria (plastosomes); but Ciaccio ignores the fact that the iron-hematoxylin stain, which he himself used, acts in much the same way.

It is quite evident that our information is not sufficiently clear-cut. The technique upon which the above-mentioned observations are based is inadequate. There has been no attempt made to estimate quantitatively either the mitochondria or the hemoglobin, and in spite of this the old presence-and-absence argument is made use of. Fixed preparations were alone studied. Now that it is not only possible, but easy, to stain the mitochondria specifically with janus green in these living blood-cells, and to count them, it ought not to be difficult to devise some quantitative colorimetric way of estimating the hemoglobin and to obtain decisive results. The information at hand does not show that the mitochondria exercise even an indirect influence upon the formation of hemoglobin. There is no topographical correspondence between the mitochondria and the deposition of chlorophyll. The formation of hemoglobin from mitochondria alone involves chemical impossibilities. It involves a change from a phospholipin into an entirely different material. Ninety-four per cent of the hemoglobin consists of the protein globin, but it also contains the coloring matter hematin. It is accordingly very much like a nucleoprotein in nature, and nothing could be further removed from the mitochondria. We ask ourselves where can the iron come from? Certainly not from a phospholipin. Pathology may help us. In chlorosis the characteristic thing is a diminution in the amount of hemoglobin in proportion to the number of red blood-corpuseles. There is either a deficiency in the formation of hemoglobin or an increase in its destruction. At any rate, the red blood-corpuseles contain less than the normal amount, and it would be interesting to find out whether there is a corresponding fluctuation in the number of mitochondria in the precursors of these erythrocytes; for if a relation exists between the mitochondria and the hemoglobin production it might be possible to detect it in this little-known condition. The mitochondria should also be studied in hemochromatosis and in other disturbances of a similar character. It is interesting to note that Policard (1912*b*, p. 230) has discovered that mitochondria form the matrix in which hemoglobin crystals are deposited in the liver of animals defibrinated by the process of Magendie. Mitochondria, however, are not associated with the deposit of other crystalloids (d'Athias, 1915, p. 68), except perhaps in plants.

SECRETIONS.

THYROID-GLAND SECRETION.

Grynfeltt (1912*c*, p. 147) was the first to suggest the formation of the colloid substance through either a direct or an indirect transformation of mitochondria. He thinks that this is quite probable in view of our general knowledge of the rôle of mitochondria in gland-cells and in consideration of some observations which he has made upon the new-born dog. He found appearances which seemed to him to indicate that the mitochondria near the surface of the apical zone of the thyroid cells undergo certain modifications and transform into clear spherules,

having some of the reactions of the colloid substance. But he admits that his observations are not sufficiently numerous to permit him to arrive at any definite conclusion. It is difficult to conceive of the direct transformation of a phospholipin into a compound containing relatively large quantities of iodine.

Goetsch's (1916, p. 132) recent work on toxic adenomata of the thyroid gland is of very great interest in this connection, for he found that there is a great increase in the amount of mitochondria parallel with the appearance of the clinical symptoms of hyperthyroidism. This may be interpreted by the adherents of the transformation hypothesis to mean that the epithelium is secreting more rapidly, that the mitochondria are increased in number for this reason, and that this is evidence that they are actually transformed into the secretion, just as Grynfeldt supposes. But this explanation is taking a good deal for granted. True, the hyperthyroidism may be due to an increase in amount of a single secretion which is produced normally, yet there is some evidence for the alternative assumption that the thyroid secretion is polyvalent and not univalent. Moreover, it is possible that the symptoms of hyperthyroidism may result from an increased rate of metabolism on the part of the thyroid epithelium and the consequent liberation in excess of products of this heightened metabolism rather than of normal secretion.

PARATHYROID SECRETION.

The evidence in favor of the mitochondrial origin of this secretion is still less satisfactory and convincing. Bobeau (1911, p. 186) bases his conclusion entirely upon a correspondence in the distribution of the mitochondria and certain lipid-like droplets in the cells. He demonstrated the mitochondria by mitochondrial methods which he does not specify and the lipid by the method of Ciaccio. He assumes that the lipid droplets constitute a precursor of the secretion, or rather the secretion itself, and that they arise by a swelling-up of the mitochondria.

CEREBROSPINAL FLUID.

Though Hworostuchin (1911, p. 232) was the first investigator to supply us with an accurate description of the mitochondria in the choroid plexus, it remained for Grynfeldt and Euzière (1912, p. 64) to make an attempt to discover their relation to the formation of the cerebrospinal fluid. They found three types of cells in the choroid plexus of mammals: (1) striated cells, containing many long filamentous mitochondria generally running from the base of the cell toward its distal portion; (2) vesicular cells, filled with small vesicles possessing clear centers surrounded by a peripheral layer of stainable substance; (3) vacuolated cells, crowded with droplets of variable dimensions. They concluded that these three represent different stages in the same process of secretion. According to them the mitochondria enlarge to form the vesicles, the vesicles change into the vacuoles, and the vacuoles discharge their contents into the ventricular system and thus form the cerebrospinal fluid.

Policard (1912*e*, p. 430), in a short paper published only a few weeks later, says that he is unable to accept this interpretation; for he is of the opinion that we have to deal with two processes, not with one. He found all stages between

the mitochondria and the small lipoid vacuoles, but he could not discover any relation whatever between these and the large vesicles which invade the whole cell; so that he is unable to agree with Grynfeltt and Euzière concerning the origin of the cerebrospinal fluid.

Ciaccio and Scaglione (1913, p. 167) question the evidence in favor of this interpretation. They call attention to the fact that Grynfeltt and Euzière used for the most part the choroid plexuses of horses killed in the abattoir. They say that horses of this kind are usually old and in poor condition and that for this reason results based upon them are unreliable.

Grynfeltt and Euzière (1913*a*, p. 198) have not met Policard's criticism, but they have answered that of Ciaccio and Scaglione by a careful study of animals of different kinds killed in a variety of ways. They found that cells of these three varieties occur in many forms of mammals, and they also discovered that bleeding and the administration of pilocarpin increases greatly the number of cells containing the large, clear vesicles. They advance this as evidence that the vesicles constitute in truth a stage in the formation of the cerebrospinal fluid. They have also (1913*b*, p. 101) extended their studies to the selachian *Scyllium canicula* among the lower vertebrates. The choroid plexus cells in this animal have a particularly well-developed striated border, which enabled them to make a detailed study of the fate of the large clear vesicles. They observed them pass through the plasma membrane and break for a moment the regularity of the striated border before discharging into the ventricular cavity. This, they urge, is strong evidence in support of the vesicular theory of the secretion of the cerebrospinal fluid and of their general contention of the rôle played by mitochondria in its formation. They do not, however, throw any further light upon the crucial question of the existence of transitions between the mitochondria and the vesicles. In a still more recent paper they make a general review of the whole problem, but contribute nothing new to the discussion.

My own studies lead me to agree with Policard, that Grynfeltt and Euzière may be dealing with more than one process and that we have no sufficient reason to believe that transitions occur between the mitochondria and the vesicles. Even should such transitions occur, we would want to know definitely whether or not the vesicles do form the cerebrospinal fluid.

SECRETION OF THE PAROTID AND SUBMAXILLARY GLANDS.

Regaud and Mawas (1909*b*, p. 220) have studied the mitochondria in the sero-zymogenic cells of the parotid of the ass and the human submaxillary. They found that there is a qualitative relationship between the amount of mitochondrial substance and of zymogen; where there is a large amount of mitochondrial substance there is little zymogen, and *vice versa*. Between these two extremes there is a complete series of gradations, and they believe these represent stages in secretion. They describe transitions between the mitochondria and the zymogen granules in the form of spherical bodies of variable size and staining reaction embedded in the substance of the mitochondrial filaments, and they formulate

the following theory of secretion: The mitochondrial filaments fix the substances which the cell takes up from the blood. At one or more points in the course of each filament there is an accumulation and elaboration of these materials, and at these points the filament swells up into spherules to which one can apply the name of "plastcs." Each plast gives rise to a grain which matures and grows little by little. Usually before the grain has acquired its definitive size and colorability the mitochondrial filament in which it is embedded becomes paler and can no longer be seen. The plast or grain is then set free in the protoplasm. At the moment of excretion there is a dissolution and the product passes in the dissolved state through the cell-wall. By this ingenious and plausible hypothesis they overcome the difficulty of assuming that the mitochondria form the secretion through actual chemical transformation.

FAT DROPLETS IN SEBACEOUS GLANDS.

Nicolas, Regaud and Favre (1912*a*, p. 203), working on human tissues, have done little beyond confirming Altmann's observation of vesicular mitochondria in the cells of sebaceous glands. Arguing from analogous appearances in other tissues, they suggest that these give rise to the droplets of fat, but they do not attempt to describe any transitions between the two and advance no evidence in support of their suggestion.

SECRETION OF SWEAT GLANDS.

Here also Nicolas, Regaud and Favre (1912*b*, p. 191) have studied the relations of mitochondria to secretion. They have found a relationship between the number of mitochondria and the number of secretion granules, but they have failed to discover any indication of the granules developing within the mitochondria as in the parotid and submaxillary glands. They emphasize the fact that in sweat glands the mitochondria are small and usually granular, and they say that even if the granules did arise within them, as they believe to be the case, it would be difficult to observe it.

SECRETION OF MAMMARY GLANDS.

Hoven (1911, p. 325) has made a careful study of mitochondria in resting and lactating mammary glands. He has arrived at the conclusion that they play a part in the formation of the different constituents of the milk. According to him, the mitochondria break up into granulations, some of which transform into grains of secretion, which give rise to the casein and the sugar; others transform into fat.

SECRETION OF THE PROSTATE.

Akatsu (1903, p. 566) gave the first clear-cut description of mitochondria, under the heading of "Altmann's granules," in the cells of the prostate. He expressed the opinion that they gave rise to granules of secretion. More recently Dominici (1913, p. 295) has worked over the entire question and has found that the mitochondria vary quantitatively with the activity of the cell. He thinks, however, that they play an indirect part in the formation of the secretion and that they are not directly transformed into it. He claims that De Bonis (1907, p. 14)

simply saw lipid droplets in the epithelial cells, and I am inclined to share his opinion on the basis of my own prostate preparations.

SECRETION IN THE VENOM CELLS OF MUREX TRUNCULUS.

Grynfeltt (1913, p. 11) found that the venom cells in the hypobranchial gland of *Murex* contain large granules of secretion antecedent (which stain intensely with picric acid and are therefore called "Boules picrophiles") as well as large and conspicuous mitochondria. He claims to have traced a genetic relationship between the mitochondria and the secretion granulations. He found large mitochondria which stain more faintly than the rest with the crystal violet in Benda's stain, and large mitochondria with a central core of material staining orange with alizarin just as the secretion granulations do. He also found that the masses with more of the central yellow-staining material possessed less peripheral coating of mitochondrial substance, and *vice versa*. He interprets these observations as follows: In the process of secretion certain mitochondria, arising through the fragmentation of chondriocentes, increase in size. Their central part undergoes a chemical transformation by which it loses its coloration with crystal violet and takes the orange color of the alizarin. This tint, at first very pale, increases more and more in intensity until finally it is identical with that of the secretion granules. He concludes that the mitochondria play the part of plasts and he regards the product as chemically a transformed mucus and the cells as goblet cells. His interpretation does not necessarily follow from his observations. On page 113 I have discussed in detail the theory that the mitochondria are plast-formers. Suffice it here to say that he has presented no evidence that the mitochondrial substance itself changes chemically into the material of the secretion antecedents. It may be, as I say, that the secretion is formed in the surrounding cytoplasm and is accumulated in the mitochondria on account of its solubility in them or for some other reason. The mitochondria may be entirely passive in the process. Neither are we justified in saying that the disappearance of the mitochondrial substance indicates that it is transformed into the secretion, for it may well be that it goes back into solution as in the parotid and submaxillary glands. Furthermore, we would like to know something about what the steps are in the chemical transformation of a phospholipin into a mucin, and it is just this that makes one so skeptical. We have not the right to say, in any of this work on mitochondria, that a substance of totally different character is formed by a chemical transformation of their substance, because it is altogether impossible to dissociate the mitochondria from their surroundings. These attempts to make definite statements are futile. We can hope only to make approximations to the truth and should bear in mind that the apparently homogeneous ground-substance of the cytoplasm probably plays the most important part in secretion as well as in all other cytoplasmic activities.

PANCREATIC ZYMOGEN.

Hoven (1910*b*, p. 349) has furnished detailed evidence in favor of the participation of mitochondria in the formation of secretion granulations in the pancreas. He records the presence of little swellings in the course of the mitochondrial fila-

ments which he calls "plastcs," and which stain like the mitochondria and secretion granules with iron hematoxylin, crystal violet, and acid fuchsin. These, he believes, change into secretion granules, since he has observed all stages of transition between the two. He is of the opinion (contrary to Regaud) that we are here dealing with an actual transformation of mitochondrial material.

Mislawsky (1911a; 1911b, p. 505), however, on the basis of very similar observations, finds no evidence of the direct transformation of mitochondria into secretion granulations. Schultze (1911b, p. 258) has also studied the mitochondria and zymogen granules in the frog, but does not commit himself regarding their genetic relationship. Champy (1911, p. 122) experimented with secretin and found that intermediary forms between mitochondria and secretion granules are more numerous during secretory activity. Laguesse (1911, p. 277) maintains that his ergastidions (mitochondria) play the part of elaborators in the production of pancreatic zymogen. G. Arnold (1912b, p. 268) claims that the zymogen granules are formed through a maturation of mitochondria, and Chaves (1915, p. 67) speaks of a physiological transformation.

Key (1916, p. 215) has experimented with the pancreas of the toad. He stimulated the gland by the injection of pancreatic secretion in some cases and of pilocarpin in others. In some experiments the injections were repeated at regular intervals for several days. He found that while this caused a discharge of zymogen granules, the mitochondria were not exhausted, but in some cases seemed actually to increase in length. He was unable to detect any difference between certain bleb-like swellings, which mitochondria possess in almost all secreting cells, and the other parts of the mitochondrial filaments, and he concluded from this that the blebs do not contain zymogen granules. Furthermore, the absence of reciprocal changes in the amount of mitochondria with variations in the cytoplasmic content of zymogen granules led him to believe that the zymogen granules are not formed directly from the mitochondria.

Scott (1916, p. 249), working in this laboratory, studied the effect of experimental phosphorus poisoning upon the pancreas of the mouse. He discovered that slight poisoning brings about a change in the mitochondria only, which lose their bleb-like swellings and their filamentous shape. The nucleus and zymogen granules show no changes. The interesting thing is that in a mild case of this sort the formation of zymogen is not interfered with. Indeed, in much more severe intoxications unmistakable evidences of further formation of zymogen granules are seen. Whole cells are frequently found crowded with them. This means that the mitochondria do not participate through their bleb-like swellings in the production of zymogen, because zymogen continues to be formed long after these swellings disappear. It can not come from the swellings, because there is none. This is another strong blow to the doctrine that the mitochondria *transform* into secretion granulations.

URINARY SECRETION.

The question of the relationship between changes in the mitochondria and variations in urinary secretion is a difficult one. It is complicated by the presence

of the so-called "bâtonnets" of Heidenhain, which resemble mitochondria in some respects but differ altogether in others (Policard, 1910, p. 225). In many cases it is hard to tell to which investigators refer. The "bâtonnets" have been known for years, and much careful work was done on their possible relation to secretion before the more elusive mitochondria were discovered. Another difficulty is encountered in establishing a normal from which to work. Analogy is a pitfall here, because there are such marked differences in the kidneys of different forms. Certain snakes and amphibians are good material because the cells, unlike those of adult mammals, contain characteristic secretion granulations like other glands. Above all it is necessary to distinguish sharply between physiological changes and pathological lesions; the latter will be considered on page 137.

Benda (1903, p. 127) makes no reference to any relationship between the mitochondria and the formation of secretion except to suggest that by contraction they draw the proximal and distal ends of the cell nearer together and thus aid in the expulsion of the secretion. To this Policard (1905, p. 382) rightly objects. Modrakowski (1903, p. 230), at an earlier date, described definite changes in the Altmann's granules (mitochondria) in experimental diuresis and suggests that they may act as condensers in the formation of secretion, but his illustrations do not show them. The distinctive features of mitochondria in the different segments of the urinary tubules have been studied by Regaud (1908c, p. 1145) and others.

Regaud (1909c, p. 1035) was the first to claim that the mitochondria play a definite part in the formation of the secretion. He worked with the kidneys of snakes and found that where the mitochondria are abundant the secretion granules are few, and *vice versa*. He records the gradual formation of the secretion granules in the substance of the mitochondria in precisely the same way as in the salivary glands.

Policard (1910, p. 272), however, working with the frog, after experimenting in many ways, remarks on the fixity of the mitochondria and describes no marked variations in them depending upon the quantity and character of the urine, though they respond very readily by fragmentation to pathological changes. He has described also (1912c, p. 450) the transformation of mitochondria into certain granulations of unknown nature in the developing human kidney. Fahr (1914, p. 120) has been able to stain secretion granulations and mitochondria differentially in the rabbit's kidney and believes that there is no relation between them.

More recently, Oliver (1916, p. 318) has studied the modifications which the mitochondria undergo in experimental diuresis. The "bâtonnets" in the cells of the proximal convoluted tubules, where the urea is secreted, lose their rod-like form and occur simply as rows of granules. She found that the urea also appears in the form of granules which are likewise arranged in rows and suggests that the secretion of urea is by means of the mitochondrial bâtonnets which act as condensers. It may be remarked that the rows of urea granules do not necessarily correspond with the rows of mitochondria; they may simply alternate with them. It may also be worth while to inquire whether phosphatids, of which we believe mito-

chondria to be composed, are able, outside the body, to pick up, condense, and concentrate urea. We desire further information on these and other points before passing tentative judgment on the possible rôle of mitochondria.

FATS.

The relation between vitellus, neutral fat, lipid, and myelin droplets and mitochondria is undoubtedly very intimate, for we have good reason to suppose that mitochondria are themselves, at least in part, phosphatids, and phosphatids are made up of phosphoric acid, fatty acid, glycerol, and some nitrogenous base like cholin. The exact chemical relationship of yolk spherules to ovovitellin is little understood, but it seems clear that, though ovovitellin is more of the nature of a nuclealbumin, it contains, nevertheless, a large amount, some say as much as 25 per cent, of the phosphatid lecithin. In recent years interest has become focussed on these phosphorized lipoids and there is an ever-growing demand for accurate information regarding them. The difficulties presented to those who attempt to study them within individual cells are very obvious and should be kept in mind during the subsequent discussion.

There are indications in the oögenesis of almost all organisms which may be taken to mean that the mitochondria are either partially or totally transformed into deutoplasmic substances, like *vitellus*. The same is true in certain cases of spermatogenesis where the male sex-cells are very large, approaching the eggs in structure. The relation between mitochondria and vitelline granules has also been studied in amphibian embryos undergoing metamorphosis.

The oft-cited observations of Loyez (1909, p. 191) are generally regarded as constituting the most convincing evidence of the direct transformation of mitochondria into vitelline globules. She was able to distinguish the following stages in the development of the eggs of *Ciona intestinalis*: (1) in the youngest oöcytes only a few granulations may be seen about the germinative vesicle and in the peripheral cytoplasm; (2) in a more advanced stage the mitochondria are disposed in linear series, in strings, throughout the cytoplasm; (3) the individual mitochondria become spherical, increase in size, and in their interior present a central and clearer area. Finally these globules fuse together, increase still further in size, and become the definitive vitelline spheres. She remarks, further, that in other closely related ascidians, like *Cynthia tetraedra* and *Cynthia morus*, the vitelline globules are not formed from mitochondria, but arise quite independently from them. These results are partially confirmed by Govaerts (1913, p. 415), working with insects, but he has not been able to demonstrate a direct transformation of mitochondria into vitelline spherules.

The clumping of mitochondria and their fusion to form fatty masses which enter into the composition of the vitellus have been described by Henneguy in the oögenesis of *Pyrrhorcis apterus*, and by Fauré-Fremiet (1910a, p. 548) in both the oögenesis and the spermatogenesis of *Lithobius forficatus*, but they do not specify the properties of the said fatty masses.

Van Durme (1914, p. 118) has gone into the question in detail from the mitochondrial point of view in birds. He describes the origin of vitelline granules by a direct transformation of mitochondria and by the initial deposition of vitelline substance in a clear vacuole and its subsequent growth.

Coghill's observations (1915, p. 349) on the relation of mitochondria to *yolk* are perhaps the most interesting, since he alone worked with living cells. His investigations point to the conclusion that the change takes place in the reverse direction, that is to say, from yolk to mitochondria. He studied with great care yolk-laden cells of amphibian embryos which he stained vitally with janus green. He observed structures actually originating from the surface of the yolk globules, by a chemical transformation of their substance, which migrated into the rest of the cytoplasm, where they became indistinguishable from the other mitochondria in the cell. Their morphology, their reactions to janus green, and their staining properties when fixed were identical with those of true mitochondria. From this he concluded that the mitochondria arise from the yolk. Without subscribing to this view, it may be said that these results are not so much at variance with those of other workers as they might at first sight appear to be; for many reactions are reversible and there is no reason why this should be an exception to the rule. Then again, the amphibian embryo is an entirely different tissue from the avian or mammalian egg and there is no reason why the processes going on in it should be identical.

The possible relationship of mitochondria to the formation of *neutral fat* has been under discussion since the time of Altmann (1889, p. 94), Metzner (1890, p. 82), and others; but Dubreuil (1911*b*, p. 264) has given the most detailed as well as the most recent account of it. The relationship of mitochondria to droplets of lipid in nerve-cells I have already discussed (1914*a*, p. 13). Reference should also be made to the work of Azzi (1914, p. 7).

OTHER PRODUCTS.

The problem of the origin of the *external segment of the rods and cones of the retina* is a very intricate one, but a few words of description will serve to make it a little clearer. It is well known that the intimate structure of the rods and cones is essentially the same, though their form differs. The constitution of the outer segment of each is alike, so far as our present methods reveal it. It is very dense, especially about the periphery, which is bounded by a sort of envelope. It frequently exhibits a transverse striation, just as if it were made up of superposed disks. Authorities are not agreed as to whether this occurs in the living condition or whether it is simply the result of the technique employed (see Mawas, 1910*b*, p. 115). A distinct longitudinal striation has been described by Ranvier in batrachians, but it does not seem to obtain in other forms. The core of this outer segment contains, according to Leboucq (1909, p. 597), an axial filament which is attached to a centrosome in its most proximal part.

There is also a close resemblance between the structure of the inner segment of the rods and of the cones, but it is somewhat more complicated than the outer segment, is less dense, and possesses more fluid cytoplasm. There is no hint of

transverse striation, but the longitudinal striation is very marked. It is difficult to analyze. Apparently several factors enter into it. In the first place, there are the fibers of Müller, which do not belong to the cell itself. The exoplasmic prolongations of the membrana limitans externa give a striated appearance. The so-called baskets ("corbeilles") also contribute to it. These are supportive. The cell-membrane itself seems, according to the best accounts, to be striated. Mawas (1910*b*, p. 117) emphasizes, in the rabbit and in man, a striation which is intracytoplasmic and which is due to the peculiar arrangement of the mitochondria in a peripheral sheet just beneath the cell-membrane. This, however, Leplat (1913, p. 220) failed to observe in birds. Leboucq (1909, p. 594) gives a detailed description of a system of intracytoplasmic filaments arising near the centrosome at the base of the external segment, at first spreading out in the inner segment to form the "ellipsoid" or "Fädenapparat," then condensing into a single filament, in the rods, and into a bundle of three or four filaments in the cones and running toward the nucleus. The neurofibrils are also to be reckoned with in connection with this longitudinal striation; little is known for certain about them. Small droplets of oil, "Ölkügel," occur in this inner segment in the early stages of development and may persist in the fully differentiated state. Small quantities of pigment are occasionally observed. Nothing is known of the Golgi apparatus in the rods and cones in the adult condition, though Cajal (1915, p. 21) has described and figured them in the early stages of development. Now that we have an idea of the elements, we can proceed with the problem at hand. It is the envelope of the external segment of the rods and of the cones that is said to be formed by a transformation of mitochondria.

Leboucq (1909, p. 593) was the first to advance this view on the basis of his observation that it stained intensely violet (like mitochondria) by the Benda method of coloration, but his paper deals more with the centrosomes and their transformations than with the mitochondria and is merely suggestive.

Mawas (1910*b*, p. 114) studied the mitochondria in the fully developed retinae of a number of vertebrates, including man (1910*c*, p. 113). He did not use any embryos. He calls attention to the fact that the external segment stains intensely with iron hematoxylin, like the myelin of peripheral nerves, and blackens with osmic acid, like fat (Ranvier). In addition, he confirms Leboucq's and Magitot's observations that it stains with mitochondrial dyes. It is soluble in alcohol and xylol. These are, he says, the reactions of mitochondria, and he therefore looks upon the external segment of the rods and of the cones as an example of diffuse mitochondrial material present in protoplasm without any structural differentiation whatever.

Leplat (1913, p. 219), extending some already published work, attacked the same problem in the developing chick with mitochondrial methods with a view to determining exactly the part played by the mitochondria. He described the heaping-up of mitochondria (first around the base of the axial filament in the external segment) and their subsequent extension along its whole length. These, he believes, form the envelope (p. 218) and he attempts to draw a close analogy between this process and the grouping of mitochondria around the axial filament

in the spermatozoon, which to my mind does not add any strength to his argument. He found that there is a chemical change in addition to the change in position of the mitochondria; for these mitochondria, which he styles "grains chromophiles," are more resistant to insufficient fixation than the mitochondria in the rest of the cell.

Levi (1914, p. 199) comes out definitely against this hypothesis, and Duesberg (1912, p. 752), on the ground of his own observations on the retinae of chick embryos, is inclined to reserve judgment on the question. According to Levi, the external segment is a cuticular formation and is not mitochondrial in nature, and he cites some earlier work, which he already published in 1901, in support of this conclusion. He worked on *Triton* larvæ and his observations do not tally with those of Leplat, yet both may be correct, because we can not take the position that the retinal elements in widely different forms originate in precisely the same way. We must try not to assume an altogether ultracritical attitude. The evidence presented appears to be fairly conclusive that, in the bird at least, the envelope of this external segment is mitochondrial in origin, for it is certainly lipoidal and chemically resembles mitochondria to some extent. We can not regard it as a direct transformation, however.

THE "RANDREIFEN" OF AMPHIBIAN RED BLOOD-CORPUSCLES.

Meves (1905, p. 103) advanced the view that this fine peripheral network, just within the cell-membrane, results from the coalescence of individual mitochondria. He based this conclusion upon the similarity which he found in the staining reactions of this network and the mitochondria by his iodine-acid and malachite-green method. This is to be regarded merely as a suggestion until the material has been worked out more carefully with adequate methods of technique.

EOSINOPHILIC GRANULATIONS IN LEUCOCYTES.

According to Ehrlich, the granulations which he described in blood-cells are a true product of the secretory activity of the cells themselves. Meves (1910b, p. 656) concludes that these granules, just like those of gland-cells, arise from mitochondria, and he forwards the additional argument that the eosinophile cells of the salamander contain few if any mitochondria, the assumption being that the mitochondria have all been transformed into the granules. It is unnecessary to point out how loose this reasoning is. I have studied the mitochondria in living human polymorphonuclear leucocytes stained vitally with janus green and have found no indication at all of transitions between the mitochondria and the specific granulations. In fact, they are entirely distinct, (1) on the basis of the high refractive index of the eosinophile granules, the low refractive index of the mitochondria; (2) the large size and spherical shape of the granules, the small size and rod-like shape of the mitochondria; and (3) the lack of coloration of the granules and the intense specific staining of the mitochondria. Moreover, I have examined the eosinophilic myelocytes in the bone marrow of the guinea-pig, both vitally stained with janus green as well as in fixed and stained preparations, without finding any trace of a transition between the mitochondria and the granules. Neither have I been able to find any indication of a transition between the mitochondria and the neutrophilic or basophilic granulations in man.

GLYCOGEN FORMATION IN THE LIVER.

Arnold (1908, p. 365) claims that mitochondria play a part in the origin of glycogen, but does not submit any evidence. Fiessinger and Lyon Caen (1910, p. 454) conclude that glycogen is formed between the mitochondria, not in them.

GRANULATIONS IN CONNECTIVE-TISSUE CELLS.

The whole Lyon school, headed by Renault, look upon cells of the connective-tissue variety as gland-cells, and they have attempted to correlate the mitochondria with the processes of secretion, which they believe to go on in them. They differ from most investigators in including lymphocytes under this heading.

Renaut and Dubreuil (1906*b*, p. 230) call attention to the following observations: (1) that the périnème (*i. e.*, the mitochondrial apparatus) is rudimentary in young forms like the small rhagioerine lymphocytes; (2) that it develops more and more in direct measure as the cell becomes older and its secretory activity grows; (3) that it decreases little by little as the cell ages and its secretory activity declines. In other words, they relate the mitochondria to the act of secretion; but they say nothing about transitions between the mitochondria and the secretion granules, which they style "grains de ségrégation." This is an important omission.

A few years later Dubreuil (1913, p. 134) brought forward more detailed observations of the same nature, bearing on the same problem. He believes that all the connective-tissue cells in the embryo are secreting, and he has found that they all contain abundant mitochondria. In the adult he considers the secreting function to be relegated to the round and mobile connective-tissue cells and to the elasmatocytes, the fixed connective-tissue cells being quiescent. He discovered that the mitochondria are very numerous in the former and rare in the latter. He cites his observations on fat-cells as a second example. While the mitochondria are few in connective-tissue cells destined to undergo fatty metamorphosis, they are enormously augmented in the early stages of differentiation, and, when the cell has accumulated the maximum amount of secretion in the form of fat, another change takes place and the mitochondria disappear almost completely. The cells of the lymph and serous fluids furnish, he believes, still another instance of parallelism between the amount of mitochondria and the secretory activity. In direct proportion as they increase in size, departing from the true lymphocyte type, their secretory properties increase; and at the same time the mitochondria also increase and soon form a dense layer about the nucleus. In addition to this, he thinks that young cartilage and bone cells secrete and for this reason contain many more mitochondria than the older ones. Lastly, he has observed that, on inflammation, fixed connective-tissue cells, which he supposes have lost their ability to secrete, begin to secrete again at a very rapid rate; and that, coincident with this, their mitochondria become just as abundant as in the actively secreting connective-tissue cells of the embryo. He admits that a demonstration of the direct transformation of mitochondria into products of segregation in large mononuclear cells, connective-tissue cells, cartilage and bone cells, has not been made, but claims that we can nevertheless believe in such a transformation because it occurs in many

other gland-cells. This conception is also in accord with the theory that the mitochondria play the part of "ecleosomes" (p. 103).

Favre and Dubreuil (1914, p. 91) have, still more recently, attacked the same problem in plasma cells. Here they find a similar relation between the mitochondria and the "grains de ségrégation." Where there are many mitochondria there are few "grains de ségrégation," and *vice versa*. They make no reference to morphological or chemical transitions between the two.

Their general conclusion of the participation of mitochondria in the secretory activities of connective-tissue cells is open to criticism along several lines. In the first place, we know next to nothing about the said secretory activities. We can not measure them or obtain any clue to the properties of the secreted substances. Yet we can not say that these cells do not secrete because, in all probability, all cells give off materials into the surrounding fluids. Consequently their statements that the secretory activity is high or low must be accepted with caution, for the mere presence of a large number of "grains de ségrégation" does not suffice. Their increase in number may be due, not to an increase in the rate of their production, but to a decrease in the rate of their elimination. We may be dealing in some cases with retention pictures. Neither do we know definitely that these "grains de ségrégation" represent the secretion. So much for the secretion itself and for their statements of variations in its amount, for their argument is none other than the old one of presence and absence and of reciprocal relations.

In the second place, investigators will question their genetic series, particularly with reference to the lymphocytes; because if the lymphocytes do not transform into the other connective-tissue cells, as they believe, there is nothing significant about their small content of mitochondria as contrasted with the large amount in the connective-tissue cells, which they suppose to have assumed the ability to secrete. That is to say, they have not established beyond question the steps in their series, in which they claim that there is a relation between the amount of the mitochondria and the secretory activity, the weak link in the chain being the lymphocyte, which many people look upon as a fully differentiated blood-cell.

Lastly, the absence of transition forms between the mitochondria and the "grains de ségrégation" can not fail to escape attention. None of these investigators claims to have observed them, and we must therefore reserve judgment with regard to the part played by mitochondria in the formation of the "grains de ségrégation" and in the production of the unknown secretion of connective-tissue cells.

CILIARY APPARATUS.

Saguchi (1917, p. 265) has made a careful study of the development of the ciliary apparatus. He describes first an accumulation of mitochondria (chondriocentes) between the nucleus and distal cell border, a subsequent migration toward the distal cell border and a transformation into rod-like corpuseles arranged in rows. These emit, at successive periods, short initial cilia, which gradually lengthen until the definitive cilia are formed. He is careful not to assert that there is a chemical transformation of the mitochondrial substance into that of the cilia.

X. PHYSIOLOGY.

There is a very widespread belief that mitochondria play an active part in cellular activity, though the nature of their behavior is almost wholly obscure. We must approach a problem so difficult with caution and take stock of the possibilities before we attempt to arrive at any conclusion. Now, vital processes, or life phenomena in the cells, which are the ultimate structural units in our bodies, are naturally divided into two great groups—those which are fundamental, being common to all cells, and those which are special, representing the peculiar duties which certain highly differentiated, older cells have learned how to perform in the course of their development.

Among the fundamental activities of protoplasm we are accustomed to group metabolism, respiration, irritability, growth, and reproduction. Young cells possess all of them as contrasted with the older, more mature cells of later stages. These older, fully differentiated cells can no longer reproduce their kind, but nevertheless contain mitochondria, so that we may safely eliminate reproduction from consideration. Irritability is the power of being able to respond in some way to variations of any kind in the environment. The stimulus varies, but the mitochondria are constant. They are no more numerous in nerve-cells in which irritability is developed to an extraordinary degree than in other cells. This leaves for consideration only metabolism and respiration (which is really a special phase of metabolism), though some would be inclined to include the "mnemonic factor" with these basic activities of life. Mathews (1915, pp. 68 and 587) refers "memory" to the cells themselves, and gives us an inkling of the possible chemical reactions upon which it may be based, though psychologists tell us that it is due to the interaction of reflex arcs rather than being a manifestation of the life of individual cells. It is highly probable that mitochondria are concerned in growth.

The differential activities, on the other hand, are secretion in gland-cells, contraction in muscle-cells and cilia, and irritability and conduction in nerve-cells. As a matter of fact they are merely the fundamental activities of living material, enhanced and intensified, for we must admit that even the most embryonic cells are capable of giving off substances, that is to say, of secretion.

SECRETION.

Ever since the work of Altmann there has been a tendency to assume that the mitochondria play a part in the formation of secretion granules. This tendency blossomed out vigorously under the influence of the more recent theoretical considerations of Meves and Regaud. Meves (1908, p. 845) thought that the mitochondria constituted, in part, the material basis of heredity and he believed that they become chemically transformed into all kinds of cellular differentiations like secretion granules, fibrils, etc. The working hypothesis of Regaud (1911, p. 685) was a little different. He at once perceived the difficulty of assuming that the phosphatid albumin mitochondrial complex becomes chemically altered into a large variety of different materials and advanced his "eectosome" theory.

according to which the mitochondria play the part of plasts, choosing out and selecting materials from the blood-stream and the cytoplasm, condensing them and changing them, in their substance, into infinitely diverse products. Chemical substances are thus supposed to be drawn in from the outside, not to be formed through a transformation of mitochondrial material. Regaud thus resolves the problem largely into one of permeability. His conception is, as he himself points out, essentially a modification of the celebrated lipoid membrane theory of Overton, the chief difference being that, according to Regaud, the lipoid substance is said to be distributed throughout the whole cytoplasmic area in the form of mitochondria instead of being confined to a layer on the surface of the cell.

Specific cases of the alleged development of secretion granulations from mitochondria have been discussed under the heading of "Histogenesis" (p. 116).

CONTRACTION.

The view that mitochondria are directly concerned with the motor activities of cells is of historic interest only. Benda (1903, p. 127) advanced it upon the basis of the following considerations: (1) similarity in the microchemical reactions of the dark bands of striated muscle and mitochondria; (2) the grouping of mitochondria about the axial filament of the developing spermatozoon; (3) the heaping-up of mitochondria about the roots of cilia in ciliated cells. Benda later (1914, p. 25) modified his conception by making the statement that mitochondria are concerned with the development of the motor organs of the cell, like myofibrils.

Holmgren's (1909, p. 307) observations on changes in mitochondria in muscular fatigue are of special interest here. He made ingenious experiments with dragon-flies, holding them by the thorax between finger and thumb and allowing them to continue beating their wings furiously for different lengths of time. He found that granules which stained in a typical way with the Benda method and with iron hematoxylin underwent definite alterations, depending upon the severity of the fatigue. The granules changed their position, diminished in number, and stained less intensely. These findings are certainly suggestive, to say the least, but they have never been confirmed. They evidently merit further attention.

Fauré-Fremiet (in discussing the paper by Regaud and Mawas, 1909b, p. 235) records the observation that the mitochondria, gathered about the contractile filament in the peduncle of vorticella, are unchanged at the moment of contraction. Regaud (1908b, p. 209) observed that the mitochondria in the ciliated cells of the urinary tubules of cold-blooded vertebrates are few in number, are distributed without apparent order, and have no relation to the cilia. Finally, Shipley (1916, p. 444) discovered that there is no relation between the motility of *Trypanosoma lewisi* and the amount of mitochondrial substance within them.

IRRITABILITY AND CONDUCTION.

Curiously enough, the problem of mitochondria in this connection has so far been untouched. This may perhaps be due to the reluctance of investigators to even admit their existence in nerve-cells (see p. 101). Accordingly, it is a very pertinent question to inquire whether there are alterations in the mitochondria in

nerve-cells in muscular fatigue. Strongman (1917, p. 169), working in this laboratory, has undertaken to answer this. White mice were selected for the experiments because they are the smallest mammals which can conveniently be used in the laboratory and because more is known of the qualitative (Thurlow, 1917, p. 37) and quantitative (Nicholson, 1916, p. 329) variations in mitochondria in their nervous system than in that of any other mammal. They were fatigued by the very simple method which Professor Tamao Saito uses, of letting them swim in water until they are exhausted. It was soon discovered that they swim more actively when the water is slightly agitated and is raised to body temperature; otherwise they soon learn to float and refuse to exercise.

The experiments were controlled in the usual manner by using only mice of known age and by examining, in exactly the same way, an unexercised mouse of the same litter for comparison. Five experiments of this kind were made, the mice swimming 1 to 2 hours continuously before they were completely exhausted. Larger mammals will swim for a day or more before exhaustion. Experience showed that young mice 25 to 30 days old are more suitable than adults, because they are more easily tired.

In each of the five experiments the fatigued mouse and the control mouse from the same litter were chloroformed. They were then fixed by the injection of a formalin and bichromate mixture in accordance with the method advised by Cowdry (1916*a*, p. 30). The brains were then removed, mordanted, dehydrated, and cleared together in the same bottle, and they were embedded in the same block of paraffin. Sections, cut 4 microns in thickness, from the fatigued and from the control, were mounted on the same slide so as to avoid variations in the staining. The sections were then stained with fuchsin and methyl green, the fuchsin coloring the mitochondria crimson and the methyl green staining the Nissl substance a bluish-green color. Preparations were made of the cortex, the cerebellum, and the spinal cord, in this way, from each of the five experiments.

The net result of Strongman's five experiments, with their controls, was to show that mitochondria are surprisingly constant in nerve-cells. A fair degree of fatigue, as well as a certain amount of fright, brought about no constant changes in them. It is quite possible that more prolonged exhaustion, if it can be induced, may lead to definite and precise alterations. It is perfectly clear that these results are in complete accord with those which Key and Scott obtained with the pancreas, because they show that in the nerve-cell, also, the mitochondria are not directly concerned with specialized activities. They do not play a part in conduction any more than they do in secretion, which strongly points to the general conclusion that they are concerned in some fundamental process common to all cells.

METABOLISM.

Here, as in all other fields of investigation, observation has preceded experimentation. Mitochondria have been carefully studied in phylogeny as well as in ontogeny. Their wide distribution is amazing. It has already been pointed out (page 72) that they occur from man to the most lowly protozoon and from the

angiosperms to the fungi, though their existence is doubtful in the myxomycetes, schizomycetes, and most of the algæ. They are apparently identical in both plants and animals (N. H. Cowdry, 1917, p. 225). They are indeed as characteristic of the cytoplasm as chromatin is of the nucleus. They differ slightly in composition just as chromatin does. The fact that they are most abundant in the active stages of life of the cell and decrease in number as the cell becomes old and senile is not without significance. We find them in the egg and in all the tissues of the developing embryo—from the very beginning (when the cells have no definite specialized activities) to the later stages and adult life, when each has assumed its own peculiar duty of secreting or contracting, or conducting, as the case may be. In other words, their presence in the absence of specialized activities indicates that, in these early days of development, they are either inert or else play a part in the fundamental vital processes. The conclusion is obvious; and since they do not differ in any noteworthy particular in the later stages, the assumption is justified that here also their function is a basic generalized one.

The meager and unsatisfactory yet direct experimental evidence at hand seems to support this view. Thus Romeis (1913c, p. 12) has found that mitochondria are very numerous in actively regenerating tissues; Busacca (1915, p. 232) found that they decreased in number with fatigue in the cells of the retina stimulated with intense light; Homans (1915, p. 12) associated the number of mitochondrial filaments with an increased activity of islet-cells in experimental diabetes; Policard (1910, p. 284) showed that there was an increase in the number of mitochondria in kidney-cells on administration of phloridzin, and so on.

These statements relate, however, only to the general impression given by the study of sections. There has been no attempt to distinguish, in a clear-cut way, between absolute and relative fluctuations in mitochondrial content. The observations have not been controlled by a careful estimation of cell-volumes. Thurlow (1917, p. 37) has been the first to realize these discrepancies. She has established a definite mitochondria cytoplasmic rate in the nerve-cell, just as Hertwig years ago measured the nucleus cytoplasmic ratio (see p. 80).

There have been no carefully checked observations on qualitative changes in mitochondria with cell activity, although we have abundant evidence that the solubilities of mitochondria vary. Of course, the changes in form of mitochondria have been subjected to careful scrutiny, but so far they have yielded little of value. The observations of Holmgren (1909, p. 308) on the changes in mitochondria in muscular fatigue are qualitative in a sense and very interesting, but they have never been confirmed.

Furthermore, processes of metabolism, as well as of respiration, are, as one would naturally expect, very easily modified in pathological states; hence the sensitivity of mitochondria to pathological change. Scott (1916, p. 243) discovered that the mitochondria are the first of all the cell constituents of the pancreas to become altered in experimental phosphorus poisoning. Moreover, the fact (which is now emerging from the numerous recent pathological studies on mitochondria) that mitochondria in different types of cells respond in much the same

way to different varieties of injurious influences—in other words, that there is nothing specific in the reactions of mitochondria to pathological change—is also in accord with the prevalent conception that they take part in a type of activity common to many cells, at which the noxious influences strike.

Relying on this confessedly inadequate information, investigators generally are inclined to believe that mitochondria participate in some of the processes involved in cell metabolism. Coghill (1915, p. 350) is rather more specific, for he relates mitochondria to the constructive side of metabolism. But the term "metabolism" is very vague. Its meaning has changed from Michael Foster's original definition. It is now used to designate a multitude of chemical reactions from the taking in of foodstuffs to the elimination of waste products, including even cellular oxidations and reductions. We have metabolism of carbohydrates, fats, and proteins to deal with. Mitochondria occur in organisms entirely independently of their diet. They are just as abundant in herbivores as in carnivores; and many of them are contained in green plants, which obtain their nitrogen from nitrates, their hydrogen from water, and their carbon from the air. Where metabolism varies in kind, they apparently do not vary. They can hardly be a stage in the utilization of a specific substance taken in in the food, much less a waste product. They must be built up in the phylogenetic scale in a variety of different ways to serve a common function. We naturally inquire whether they are reserve products or whether they play an active part in cell metabolism.

The experiments of Russo (1912, p. 203) would seem to indicate that mitochondria are reserve food products. He claims that their number may be increased in the ovarian eggs of chickens fed with lecithin. His conclusions receive some indirect support from the work of R. Van der Stricht (see p. 84), but his experiments have never been repeated, in spite of the great interest which attaches to them. We are very much in need of information along these lines and it seems that experiments on the effect of inanition and of different diets on the mitochondria in mice might yield valuable results. The monograph by Champy (1911, p. 146) on the behavior of mitochondria in intestinal epithelial cells would serve as a good point of departure.

Regaud (1911, p. 685) thinks that mitochondria play actively the part of plasts, choosing and selecting material from the surrounding protoplasm and from the blood-stream, condensing them and converting them, in their substance, into diverse products. He likens them to the hypothetical side-chains of Ehrlich, and his idea is an extension of the famous lipoid membrane theory of Overton; in other words, he believes that they function in constructive metabolism. They may act as a sort of vehicle or medium in which chemical reactions take place.

RESPIRATION.

Kingsbury (1912, p. 46) was the first to suggest that mitochondria play an important part in protoplasmic respiration. He says that osmic acid, potassium bichromate, and formalin, which are the chief ingredients of mitochondrial fixatives, are preeminently oxidizers and that their efficiency depends on the presence

of reducing substances in the cytoplasm. These he believes to be the mitochondria on account of their lipid characteristics.

But it remained for Mayer, Rathery and Schaeffer (1914, p. 619) to furnish detailed evidence in support of this view. They point out: (1) That mitochondria are chemically well adapted to function in oxidations and reductions; they are phosphatids, and phosphatids have the power of auto-oxidation (Mathews, 1915, p. 97), though some (Bayliss, 1915, p. 592) think that they do not possess it to any appreciable degree; according to Mayer, Rathery and Schaeffer, mitochondria contain unsaturated fatty acids with ethylidene groups. These have a great affinity for oxygen and oxidize themselves to aldehyde. (2) That agents which attack lipoids (like alcohol, ether, and chloroform among the anesthetics) at the same time cut down the respiratory oxidations. (3) That mitochondria are present in all cells and that respiration is the most fundamental property of living matter, etc.

Still more recently the Lewises (1915, p. 393) have arrived at much the same conclusion from their studies on mitochondria in tissue cultures. It is in accord also with my own observations on the staining of mitochondria with janus green and related dyes (1916*a*, p. 429).

XI. PATHOLOGY.

Much more work has been done on mitochondria in pathological conditions than is generally realized. It has been done from many points of view; it is widely scattered, and some of it is very difficult of access. Moreover, it is not entirely a new development, as many people suppose. We have to do with two mitochondrial literatures, an old and a new. It is the old one which is so frequently ignored, an outgrowth of Altmann's remarkable researches at Leipzig from 1880 to 1890; it flourished for a while but was soon choked by the active criticism which his views excited, for he thought that his "bioblasts" were the final ultimate living particles embedded in protoplasm which he considered to be lifeless.

The central thought which underlies all the recent work on mitochondria in pathological conditions is the conviction that we now have at our disposal a new criterion of cell activity and cell injury. We do not know it or understand it, but it has been proven over and over again in the last few months to be of great and surprising delicacy, for it responds (even before the nucleus) to injurious influences; and it has the rare merit of being cytoplasmic. We may expect environmental changes to act on it which would make no impression at all upon the nucleus. It is convenient to our hands in all cells and there is no knowing what story it will tell when we ask it.

In the study of mitochondria we are at once, and very forcibly, reminded of bacteria. One has a feeling, in looking over mitochondrial preparations for the first time, that aseptic precautions should have been taken in removing the tissues. We can easily forgive Altmann for thinking that they were elementary organisms, for the similarities between them and bacteria are really remarkable. Their form

is granular, like cocci; or rod-like, like bacilli; or filamentous, like certain vibrios; while the tendency for mitochondrial granules to become arranged in rows is strongly suggestive of streptococci. They are lipoidal and they agglutinate in the most remarkable way, under certain conditions, just as bacteria do. Fortunately, they may be easily distinguished from bacteria by their staining reactions (particularly to janus green), by their occurrence in almost all cells, by their behavior, and by their lack of independent motility. When less was known about mitochondria it was not so easy to identify them as it is now. In consequence, we have to be on the lookout in the literature for descriptions of mitochondria under the heading of "Bacteria and intracellular parasites" and *vice versa*.¹

For convenience I have indicated below the work which has already been done on mitochondria in pathology, because the space will permit of detailed discussion of only the more important contributions:

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| Adenoma of thyroid, Goetsch (1916, p. 132). | Inflammation, connective tissue, Dubreuil (1913, p. 138). |
| Anemic necrosis, Israel (1891, p. 310). | Irritation of cortex, Collin (1914, p. 592). |
| Asphyxiation in tissue culture, Champy (1914, p. 320). | Liver extirpation, effect on mitochondria in kidney, Policaud (1910, p. 245). |
| Autolytic changes, Dannehl (1892, p. 485). | Myelogenous leukemia, Klein (1910, p. 407). |
| Beri-beri, Clark (1914, p. 92). | Pernicious anemia, spleen, Shipley (1915, p. 75). |
| Carcinoma: | Phosphorus poisoning, Lubarsch (1897, p. 639); Scott (1916, p. 237); Mayer, Rathery and Schaeffer (1914, p. 607), etc. |
| Beckton (1909, p. 191). | Pigmentation, Barratt (1913, p. 566). |
| Porelli-Titone (1914, p. 237). | Poisons, various, Mayer, Rathery and Schaeffer (1914, p. 607). |
| Favre and Regaud (1913, p. 688). | Polypus of nose, Benda (1899a, p. 380). |
| Lubarsch (1897, p. 640) and others. | Prostatitis, Dominici (1913, p. 295). |
| Cicatrization in tissue culture, Champy (1914, p. 368 ¹). | Radium, Beckton and Russ (1911, p. 99). |
| Cloudy swelling in kidney, Schilling (1894, p. 470). | Regeneration, Romeis (1913c, p. 1); Torraca (1914a, p. 539; 1914b, p. 459). |
| Diabetes, Homans (1915, p. 16). | Rodent ulcer, Regaud and Favre (1912, p. 329). |
| Diphtheria toxin, d'Agata (1913, p. 443); Dibbelt (1914, p. 119). | Sarcoma treated with X-ray, Regaud (in discussion of Laguesse) (1912b, p. 111). |
| Diuresis, Policaud (1910, p. 272). | Scharlach R, skin proliferation, Barratt (1913, p. 566). |
| Edema of prepuce, Regaud and Favre (1912, p. 330). | Suprarenal gland after ovariectomy, Mira (1912, p. 43). |
| Epithelioma, G. Arnold (1012a, p. 283); Favre and Regaud (1913, p. 688). | Toxins, Mayer, Rathery and Schaeffer (1914, p. 609). |
| Fatty degeneration, kidney, Ophüls (1907, p. 136). | Tubercle giant cell, Champy (1911, p. 154). |
| Fatty infiltration, Altmann (1889, p. 94). | Uranium nephritis, Oliver (1916, p. 306). |
| Feltphanrosis, nerve-cells, Biondi (1915, p. 224). | X-ray, Beckton and Russ (1911, p. 99). |
| Galls (nematode) in plants, Nemece (1910, p. 166). | |
| Hemoglobinuria, kidney, Barratt (1913, p. 566). | |
| Hypertrophic tonsils and adenoids, Alagna (1911, p. 27). | |
| Inanition, Russo (1910, p. 173). | |

GLANDULAR SYSTEM.

Most of the work has been done on glands because of the ease with which they can be observed and experimented with. The two outstanding contributions are those of Homans on diabetes and of Goetsch on diseases of the thyroid.

Homans (1915, p. 16) produced various degrees of diabetes in the dog by removal of portions of the pancreas and by feeding with carbohydrate food. He found that the mitochondria in the B cells of the islands of Langerhans become accentuated, fuse to form droplets, and finally disappear in stages of activity, exhaustion, and degeneration of the tissue, while the other cells show no changes

¹ The relations, if any, which are affected between mitochondria and the invading bacteria have never been studied. Yet it is unlikely that two materials of similar size and shape and lipoidal properties would not act on each other in some way. Since mitochondria undoubtedly agglutinate, it is possible that they may be associated in intracellular bacterial agglutination. The difficulty of staining them both differentially would not be great and it would be interesting to compare the active invasion of virulent bacilli with the phago cytosis of nonvirulent or dead organisms.

whatsoever. According to Homans, this remarkable association of changes in the B cells with the condition of diabetes is causal, and indeed this would seem to be the most likely interpretation, though possibly they may be simply the result of the condition. In any case the work constitutes a definite contribution to our knowledge of diabetes on the one hand, and of mitochondria and cell physiology on the other.

Goetsch's work on the thyroid (1916, p. 132) is of particular interest. He has studied colloid goiters and has found that there is an actual correlation between the clinical symptoms and the amount of thyroid tissue presenting evidence of thyroid activity by the abundance of mitochondria throughout. That is to say, in cases of colloid goiter, without any symptoms of hyperthyroidism, the mitochondria are generally reduced in number to the point of being almost entirely absent. Those present are smaller than normal and there is a considerable increase in the amount of fat. In the cases of adenoma with hyperthyroidism, the cells of the adenoma are enormously rich in mitochondrial substance in the form of granules and short, thick rods, and contain no fat. In every instance of exophthalmic goiter the thyroid gland has been found to contain a great abundance of mitochondria, much in excess of the normal amount and with little or no fat. In fact, the correlation of the clinical manifestations of hyperthyroidism and the mitochondrial picture is such that Goetsch is justified in saying that the two are definitely related. His conclusions are based upon the study of approximately 125 cases of thyroid disease in the human.

BLOOD VASCULAR SYSTEM.

The blood offers a unique opportunity for the study of mitochondria because they can so well be seen in the living cells by staining with janus green as well as in fixed smears stained by appropriate methods. Nevertheless, investigators have contented themselves with merely describing the mitochondria in the normal cells. Considerable attention has been paid to the mitochondria as possible indicators of the genetic relationship of blood-cells to each other. Blood from cases of anemia and leukemia has been studied simply because it contains cells which are normally restricted to the bone marrow and which can not be obtained under normal conditions without an operation or early autopsy. The question of the part played by mitochondria in the development of the specific granulations in leucocytes has been touched on (p. 126), but nothing whatever has been done on the changes in the mitochondria in blood diseases in man or in different experimental conditions in animals.

URINO-GENITAL SYSTEM.

The kidney has been very carefully studied and the part played by the mitochondria in the formation of urine carefully worked up, for which see page 126. Little, however, has been done on pathological changes in the kidney.

Takaki (1907, p. 250) has made a study of autolytic changes by Altmann's method. It is questionable whether Pizzini's (1908, p. 108) observations relate

to mitochondria at all. Enderlen (1908, p. 208), Hirsch (1910, p. 168), and De Giacomo (1911, p. 223) have all studied compensatory renal hypertrophy and record concurrent increase in granulations which are probably mitochondria. There is also a general consensus of opinion to the effect that mitochondria fragment on the approach of degeneration. The work of Cesa-Bianchi (1909, 1910) and Hjelt (1912, p. 207) is important in this connection.

Though much has been done on mitochondria in cloudy swelling, particularly in the kidney, little if anything has been added to the excellent account of mitochondria under the heading of "Altmann's granules" given by Schilling (1894, p. 478) and usually ignored. Schilling produced cloudy swellings in rabbits by ligation of the renal vein and found that the mitochondria lose their characteristic staining reaction and serial arrangement and decrease in number. He rightly maintains that the mitochondria are quite distinct from the albuminous granules of cloudy swelling, and he attributes the disappearance of mitochondria to the approach of degeneration. His conclusions have been confirmed by Lubarsch (1897, p. 631) and many others. Recent literature has been reviewed by Ernst (1914, p. 81).

Dominici (1913, p. 295) has studied the relationship of mitochondria in normal and hypertrophied human prostates and has concluded that they are not directly concerned in the formation of the secretion.

RESPIRATORY, MUSCULAR, AND SUPPORTIVE SYSTEMS.

The respiratory, muscular, and supportive systems have not been studied to any extent. We merely have the observations of Dubreuil (1913, p. 134) on inflammation in connective tissue already referred to on page 127.

NERVOUS SYSTEM.

Luna (1913*g*, p. 415) in a brief note records the behavior of mitochondria (plastosomes) in transplanted ganglia. They first swell up into large granules, and he claims that the cells must maintain their vitality owing to the presence in them of well-developed mitochondria. He also experimented by cutting peripheral nerves and found that the mitochondria in the corresponding ganglion cells lose their regular distribution, increase in volume, take on a more intense stain with hematoxylin, and finally disappear entirely. Clark (1914, p. 92) observed that the mitochondria are surprisingly constant and show no changes in experimental beri-beri, and Biondi (1915, p. 232) has made a study of the relations of mitochondria in autolyzing nervous tissue.

G. F. McCann (1918, p. 36) has made a study of mitochondria in the spinal ganglion cells of monkeys in experimental poliomyelitis. She has found that the mitochondria are surprisingly resistant, occurring even in those cells which no longer contain typical Nissl substance. Evidently, therefore, experimental poliomyelitis, like experimental beri-beri, does not modify to any great extent the vital processes, whatever they may be, in which the mitochondria are concerned. This is important because it brings us face to face with the fact that, while the mitochondria

are extraordinarily sensitive to some pathological changes, they are equally resistant to others. It is unsafe at present to hazard even a tentative explanation. Why this should be remains one of the great problems of pathology.

The small amount of work on the pathology of mitochondria in the nervous system is not due to a lack of interest, but rather to a misunderstanding and exaggeration of the difficulties involved. Experience has shown that the older osmic-acid-containing fixatives are almost useless for the study of the central nervous system on account of the large number of medullated and non-medullated fibers offering insurmountable barriers to their penetration. It is not generally appreciated that this difficulty may be in large measure overcome by the application of Regaud's mixture, which consists of formalin and potassium bichromate. Excellent results may also be obtained by mordanting tissues in bichromate which have been previously injected in the ordinary way with formalin.

It is important to note also that the mitochondria in the nervous system do not undergo autolytic changes immediately after death as they do in gland-cells. It is by no means necessary that the tissue be fixed while the body is still warm; 6 or 8 hours after death is often soon enough.

While we are still ignorant of the normal appearance of mitochondria in the human brain, isolated observations of their relations in pathological conditions are of but little value. Great difficulties attend the production of lesions in the brains of experimental animals, without surgical intervention, because the changes in the other organs often prove fatal before the nervous system has been affected; but in a field so promising these difficulties will probably be speedily overcome. Mitochondria, being phospholipins, differ sharply from the Nissl substance, which is nucleoprotein in nature. Accordingly, they will serve as clues to a different type of activity and will yield valuable information upon the question of nerve-cell physiology. From their chemical constitution also it is probable that on disintegration they may elaborate cholin, and inasmuch as organic diseases of the nervous system can be separated from functional neuroses by the formation of cholin in the one and not in the other (Halliburton, 1907, p. 74), it is quite likely that a study of mitochondria may afford a cytological basis of distinction between these two great groups of nervous diseases.

REGENERATION.

Romeis (1913a, p. 10) has made a study of regeneration in the tails of *Triton*. In the connective-tissue cells the mitochondria become greatly increased in number and he finds all the stages in the formation of fibrils reported by Meves. The same is true in muscle-cells, and the mitochondria also increase in number in the glands of the skin and in bone. He believes that with regeneration the cells become more embryonic with resultant increase in mitochondria and that the various structural differentiations are formed by the mitochondria and the cytoplasm acting together.

Torraca (1914a, p. 539; 1914b, p. 459; 1916, p. 326) has made a number of contributions to the subject, working on cartilage, striated muscle, and glands.

He also describes an increase in mitochondria in the active stage of regeneration and is of the opinion (with Romeis) that the mitochondria participate directly in the formation of secretion.

It is accordingly interesting to note that Oliver (1916, p. 307) describes a distinct *decrease* in the number of mitochondria in regenerating kidney-cells in chronic uranium poisoning, but this discrepancy may be due to studying a different stage in the regenerative process.

FEVER.

The single observations of Policard (1912*d*, p. 229) on the temperature solubility of mitochondria are perhaps significant. He found that exposure for 30 minutes to a temperature of from 47° to 50° C. dissolved the mitochondria in kidney-cells without affecting the appearance of the nuclei. It is well within the bounds of possibility that a prolonged or intermittent temperature of say 40° C., as in a high fever, may bring about a solution or chemical alteration of mitochondria in some cells of the body. This is, at any rate, an interesting thought in connection with Welch's (1888, p. 403) belief that fatty degeneration in heart-muscle is in some way associated with high fevers.

ACIDOSIS.

Thus far no account of mitochondria in acidosis has appeared. Now, it is common knowledge that mitochondria are very sensitive to acids. It is also well known that one of the first manifestations of acidosis is a marked inhibition of the respiratory oxidation of the cell (Mathews, 1915, p. 247). If there is anything in the theory that mitochondria function in processes of oxidation and reduction it is possible that these two facts may be related. Let us remember also the dyspnoea in acidosis. Moreover, mitochondria respond to a wide range of noxious influences by swelling up before going into solution, which might well be due to the effect of increased H-ion concentration upon their protein fraction, causing it to become hygroscopic and to swell. The affinity of injured cells for basic anilin dyes is probably due to a swing of the reaction in them toward the acid side.

TUMORS.

The older pathologists did not touch on the question of mitochondria in tumor-cells. Veratti (1909, p. 34) and Beckton (1909, p. 182) independently, in the same year, published their researches on tumors. Veratti applied the uncertain Golgi method to cells of a transplantable mouse carcinoma and brought to light filamentous structures which appeared to be mitochondria. Beckton's technique was a little better, though he relied entirely upon the old, original Altmann's method, but his conclusions were startling and stimulated a great deal of interest. He thought (1909, p. 191) that the granules of Altmann (mitochondria) were absent in the cells of malignant tumors and that malignant growths could be distinguished from benign in this way. Bensley (1910*a*, p. 81) at once grasped the possible importance of this assertion and proceeded to test it out. His results were entirely at variance with those of Beckton, for he found that as a matter of

fact mitochondria are just as abundant in rapidly growing tumors as in those of the benign variety. This has been confirmed by G. Arnold (1912*a*, p. 283), Favre and Regaud (1913, p. 688), Porcelli-Titone (1914, p. 237), and all the others who have worked with malignant growths.

A number of purely descriptive papers have appeared on mitochondria in different varieties of tumors, chiefly in human tissues taken at operation or autopsy. No results of great importance have been achieved (see p. 135). Let us hope that the work will take an experimental turn. Much remains to be done. One is tempted to entertain, as a working hypothesis, the view that mitochondria may serve, in a measure, as indicators of the effect of X-rays, radium, and other so-called therapeutic agents on tumor cells. The isolated notes on mitochondria in human tumors are all very well to begin with, but we must have detailed and comprehensive accounts of mitochondria in some particular type of tumor in animal cells before experiments can be profitably commenced. We want to know the whole story of mitochondria from the origin of the tumor to the end. Another prerequisite is some accurate information upon the effect of X-ray and radium upon normal cells, because it appears that the results of Beekton and Russ (1911, p. 105) require confirmation.

It would be interesting to compare the mitochondria in the cells of the crown gall in plants with cancer cells in man to ascertain whether they present any points of similarity or dissimilarity. The degree of resemblance between the two is of prime importance in view of Erwin F. Smith's (1917, p. 277) discovery that crown gall is caused by an infection with a specific organism and his suggestion that cancer in man is likewise infectious. Moreover, tumors offer a new and attractive field for the study of the behavior of mitochondria in histogenesis. Nobody has even touched on the relations of the myofibrils in myomata, and of the connective-tissue fibrils in fibromata to mitochondria, to say nothing of the fascinating problems presented by the rarer types of neuromata. If there is such a thing as dedifferentiation in tumors, a study of the process might go far toward clearing up the whole problem of the part played by mitochondria in normal differentiation, for many chemical reactions are reversible; if the mitochondria form the large variety of chemical substances which it has been claimed they do in normal differentiation, then it is quite reasonable to suppose that they may be reformed from these same materials, dedifferentiation taking place.

XII. DISCUSSION.

THE GENERAL RESULTS OF MITOCHONDRIAL WORK.

(1) It constitutes a definite addition to our knowledge of the fundamental structure of living material, for we have found in them a definite and concrete class of cell granulation presenting even less variation than the chromatin and distributed through almost all living matter.

(2) The discovery of their fatty, phosphatid nature (just when physiological chemists are becoming interested in fats, whereas formerly their chief attention was devoted to the study of proteins, owing to the inspiration of Emil Fischer's work on protein synthesis), is a coincidence of some importance because it makes probable a rapprochement between cytological and chemical work, a new and promising point of contact having been established.

(3) The fact that they do serve, in some cases, as a basis for cell classification may prove to be of great value when followed up. This is particularly true in questions of cell genealogy. The differences, however, in the appearance of mitochondria in cells of different type are usually but slight, and this is not to be wondered at in view of the generality of their distribution in all protoplasm and in view also of the probability that they play their part in some fundamental activity common to many cells, not in highly specialized functions likely to differ from cell to cell.

(4) In embryology they have excited the greatest interest. The radical claims concerning their rôle in histogenesis have forced the reinvestigation of the entire field. There is much difference of opinion, but it can be safely said that they are associated in the formation of certain substances like fat, lipid, pigment, and perhaps also secretion granulations. While it seems clear that they do not form these substances by direct chemical transformation, the exact part which they play is a mystery and will probably remain so for some time to come. In inheritance also much has been done, but no good experimental evidence has been found in support of the supposition that they act as carriers of heredity.

(5) With regard to the part which they play in the physiology of the cell, we have learned only that it is fundamental rather than specialized. We suspect that they are concerned either directly or indirectly with processes of metabolism or protoplasmic respiration, and this is as far as we can go.

(6) The extraordinary sensitivity of mitochondria to pathological change has already proved of the greatest value in experimental medicine. It has been found that, in some cases, they are much more sensitive than the nucleus. Preparations made by the old methods designed to give nuclear detail show no trace whatever of the more subtle changes which may be brought to light by the newer mitochondrial technique. Furthermore, since the mitochondria are so different from the nucleus in every respect, it is not surprising that they have proved themselves to be indicators of an entirely different type of activity. It is in fact this quality which has been of such great service in the study of exophthalmic goiter (p. 136).

(7) In botany the study of mitochondria has greatly advanced our knowledge of the development of plastids. It has been found that the plastids arise from the

mitochondria; in other words, that mitochondria are associated in the production of starch and chlorophyll and a great variety of other substances.

(8) And finally, their intensive study in so many cells of both animals and plants has resulted in a well-marked movement toward the study of the whole cytoplasm and many facts of importance, not directly related to mitochondria, have been brought to light. It has forced, for instance, a complete readjustment of our conception of protoplasm.

(a) *Flemming's filar theory.*—According to this hypothesis, protoplasm consists fundamentally of a homogeneous ground-substance in which fibrils are embedded. The relatively dense and refractile fibrils he called the *mitome* and the watery fluid between them the *paramitome*. The idea has been much modified by himself and others. Now that we are in a better position to understand his *fila*, we realize that they are a very heterogeneous group of structures. Many of them are artifacts and others mitochondria. He included under the same heading such widely different structures as spindle fibers. This mere separation of protoplasm into *mitome* and *paramitome* helps but little.

(b) *Altmann's bioblast theory.*—Altmann observed (in many varieties of cells) minute granular rod-like and filamentous structures which he took to be elementary organisms. He thought that they existed in the form of colonies in cells and that they multiplied by division. He formulated the statement *omne granulum e granulo*. According to him they constitute the vital living substance as contrasted with the lifeless inert ground-substance containing them, in token of which he called them "bioblasts." Recent work on the bioblasts has robbed them of all their mystery. They are in reality a heterogeneous class of cell granulations like Flemming's *fila*, no more living than the rest of the cytoplasm and comprising mitochondria for the most part, but some fat, pigment, and secretion antecedents in addition. We can see now Altmann's many mistakes as well as appreciate the element of truth in his conception, for mitochondria are in reality almost universal constituents of protoplasm.

(c) *Fromann's reticular theory.*—In terms of this hypothesis all protoplasm consists, in the last analysis, of a relatively dense reticulum of fine threads, sometimes called spongioplasm, and of a more fluid material in the interstices called hyaloplasm. Contractility of the threads has been invoked to explain movement. Others think that they constitute the origin of fibrillar structures, like myofibrils. Recent studies in cell dissection by Kite and Chambers have failed to reveal the existence of such a reticulum and our knowledge of mitochondria is incompatible with its existence, for we find that they move freely from place to place in the cytoplasm without let or hindrance. Nevertheless the conception persists in our text-books in the form of misleading diagrams which should be eliminated as quickly as possible.

(d) *Butschli's foam theory.* Butschli afforded strong experimental evidence in support of the alveolar theories of the structure of protoplasm, according to which the continuous fundamental substance is composed of alveolar walls and alveolar contents. The foam structure is sometimes visible in the living condi-

tion and sometimes it is not. Such a formation would develop great surface tension. Butschli is to be credited for the first attempt to explain the phenomena of mitosis in terms of physics and chemistry. Apparently he was right in explaining certain kinds of amœboid motion on the basis of surface tension, though he believed that the motion took place through the local enlargement of the alveoli. In spite of many criticisms this theory is quite stimulating and helpful in the study of cell physiology.

These theories are not so clear-cut as they appear to be. Many amendments and subsidiary hypotheses with all grades of meaning have been introduced which we have not time here to consider. The attempts to generalize have not been fruitful. We constantly meet with filamentous, granular, and net-like appearances in protoplasm, but they are transitory and superficial. The theories go so far and no further. In my opinion they do not even touch on the main point at issue. They do not in any way help us to understand the nature of vital processes or the special phenomena of polarity and bilaterality in the cell, and the reason is not far to seek. It is because cytologists are usually versed in the use of the microscope and fail to realize that in protoplasm the most important things are the things unseen. Accordingly, attention has been paid to only the visible constituents and the rest have been ignored. It must be admitted that cytologists as a whole, with present-day equipment and training, are not fitted as the biochemists are for the study of the most fundamental of problems, the nature of life.

Vital phenomena are totally incomprehensible unless there exists some structural organization in protoplasm. Cellular polarity and bilaterality must depend upon it. Cells are the unit structures in our bodies, but each and every one of them is a complicated and highly organized unit. Each is a little factory which quickly brings about chemical changes, possible only in rare instances outside of the body, slowly with the aid of considerable temperature and pressure and much complicated machinery. That the cytoplasm is organized locally just as the great factory is organized in space is evident from the fact that when it is thoroughly mixed life is no longer possible. It has been proved over and over again that this organization does not reside in the visible constituents of the protoplasm, because their distribution can easily be altered without modifying either the polarity or the bilaterality, as the case may be, or disturbing to any great extent the vital processes going on. Mitochondria, pigment, and secretion granulations, fat, lipid, and all the other formed bodies are relatively unimportant. It is to the optically homogeneous ground-substance that we must look, and our microscopes will help us not at all. We must extend our conceptions to include a morphology of the ultramicroscopic and invisible; otherwise we fail.

The intensive study of the mitochondrial constituents of protoplasm has brought us an important point of contact with recent advances in chemistry. In cytology as in physiology the mechanistic philosophy is the only fruitful one. The old giant molecule or biophore hypothesis of Ehrlich is being rapidly discarded and we are beginning to entertain the entirely opposite view that vital phenomena are due to the orderly interaction of relatively simple substances, often of inorganic

nature. The growing interest in ionization, hydrogen-ion concentration, the rôle of inorganic salts, of calcium, and so on, all point in this direction. Instead of looking upon the more solid constituents as the most important, we now regard them as the least. Attention is being directed toward the phospholipins, which occur in protoplasm as a diffuse invisible deposit as well as in the form of mitochondria. According to Mathews (1915, p. 88), they are the most important substances in living matter, "for they are found in all cells, and it is undoubtedly their function to produce, with cholesterol, the peculiar semifluid, semisolid state of protoplasm. This latter holds much water in it, but does not dissolve. Indeed, it might be said that the phosphatids with cholesterol make the essential physical substratum of living matter."

That the substratum is more or less fluid is shown by the free movement of granules and other visible constituents. While many vital phenomena depend on enzymes and we know that they diffuse but slowly, so that it is not essential to assume the existence of compartments to localize their action, there must be some organization in protoplasm of an exceedingly labile sort. It is likely that this organization depends upon the pattern of colloidal structure, upon phase differences and transitory and permanent membranes, which make possible the combination and separation of chemical reactions, the orderly sequence of which is at the root of all vital phenomena; and it is this plastic framework which enables the cell to perform its proper functions, in the same way as the bones, connective tissue, membranes, and so on, permit of integration and division of labor in the body itself and control the form and function of a wonderful mechanism handed down from antiquity.

THE POSSIBILITIES OF FURTHER STUDY.

Obviously the investigation of mitochondria is rapidly progressing beyond the purely descriptive stage. The mere discovery of mitochondria in some new genus or species excites but little interest, because we have a very shrewd suspicion that they are present there anyway. It would be a great mistake, however, to assume that nothing further remains to be done. We know nothing whatever of mitochondria in any of the peripheral sense-corpuscles (p. 52), and many other problems suggest themselves. In descriptive work we can profitably let nature be the experimenter and select those forms which aid in the solution of definite questions. For instance, we can direct our attention toward the effect of environmental and other conditions on mitochondria.

Temperature.—It has been found that mitochondria go into solution when the temperature of the tissue containing them is raised for a few minutes to 45° or 50° C., which is most suggestive with regard to their chemical constitution, the effect of burns, the pathology of fever, and other questions. In this connection the study of succulent plants, like *Sempervivum*, whose internal temperature is said to reach about 52° C., in response to changes in the environment (Jost, 1907, p. 44), as compared with others growing in cold climates and in the depth of winter, would afford a new avenue of approach. The flora of hot springs should also be studied.

Atmospheric pressure.—The effect of variations in atmospheric pressure on mitochondria has not been studied on account of the technical difficulties; for this reason a comparison of their relations in alpine and deep-sea fauna might yield interesting results.

Osmotic pressure.—The effect of variations in osmotic pressure on mitochondria might be studied by comparing the mitochondria in fresh-water forms with those inhabiting the most concentrated brine. In plants it is not difficult to find a great variation in osmotic pressure.

Acidity.—One of the most characteristic properties of mitochondria is their solubility in mixtures containing even a small amount of acetic acid (0.5 to 2.5 per cent). It would be interesting to study their relations in vinegar eels, which normally live in an environment containing 4 per cent of acetic acid. The salivary glands of the rock-boring mollusc *Dolium galea*, which secretes sulphuric acid in a concentration of 4 or 5 per cent (see Bayliss, 1915, p. 359), and the acid-forming cells of plants would also repay investigation. If Macallum (1908, p. 628) and others are right (which seems, however, unlikely) in assuming that the hydrochloric acid of the gastric juice is formed in the parietal cells, then these cells must contain hydrochloric acid in much higher concentration than the juice (0.3 to 0.45 per cent), and Regaud's (1908a, p. 18) assertion that they are devoid of mitochondria becomes of vital importance.

Water-content.—The effect of variations in the water-content upon the form of mitochondria has never been determined. For this purpose the *Scyphozoa*, with a water-content of 99 per cent, and the *Trochelminthes*, which can survive prolonged desiccation (Parker and Haswell, 1897, p. 309), offer an excellent opportunity.

Hibernating animals take no water, though they continue to excrete urine. All of the water which is absolutely necessary for the continuance of their vital processes is metabolic, being produced by oxidation of the proteins, fats, and carbohydrates of the tissues through respiration (Babcock, 1912, p. 170). The general slowing-up of metabolism, the drowsiness and sleepiness, is in all probability due to a reduced water-content, with consequent retardation of all chemical reactions. The fact that the fats yield more water than either the proteins or the carbohydrates, in the case of some fats even more than their own weight of water, would seem to indicate the possibility of there being some change in the mitochondria (which are, themselves, phospholipins containing glycerol and fatty acids among other things). One would expect a diminution. The well-known occurrence of other cytological variations in nerve-cells during hibernation would also indicate that a study of mitochondria in this condition might give valuable results. Furthermore, since mitochondria are present in almost all the tissues of all animals, we must entertain the possibility that they may be in part the source of metabolic water in general. For this reason investigations into their relations in the common clothes moth, *Tinea pellionella*, desert animals like serpents and prairie-dogs, and sea-birds which have no opportunity to drink fresh water, should be undertaken.

Respiration.—Still more recently it has been claimed that the chief function of mitochondria is protoplasmic respiration (p. 133). There seem to be several ways by which this hypothesis can be tested:

(a) We may inquire whether there is any relationship between the number of mitochondria and the respiratory exchange of different organisms. Table 4 is a portion of a table compiled by Krogh (1916, p. 148) relating to certain insects. It shows a tremendous variation from as much as 82 to 1.45 calories per kilogram per hour. Yet a comparison of the mitochondria in the two has not been made.

TABLE 4.—*Metabolism of cold-blooded animals at about 20° C.*

Name.	Weight.	Temperature.	Calories.	Determination.	Authority.
		°C.			
Tenebrio larva.....	0.1	18	1.45	O ₂	Thunberg.
Formica.....	0.01	20	2.5	O ₂	Slowtsoff.
Apis mellifica.....	0.09	20	82.0	O ₂	Parlion.
Musca.....		20	24.0	O ₂	Do.
Musca.....		20	15.0	O ₂	Battelli and Stern.
Musca larva.....		20	6.2	O ₂	Do.
Bombyx larva.....	1.5	20	3.5	O ₂	Regnault and Reiset.

(b) Similarly for the oxygen consumption of tissues (Bayliss, 1915, p. 612):

Lungs, 0.015 c.c. per gram per minute.

Submaxillary gland, 0.027 to 0.089 c.c. per minute.

Suprarenal gland, 0.045 c.c. per minute.

The oxygen consumption of the submaxillary gland is about twice that of lung-tissue and the suprarenal four times. Roughly speaking, it is true that the mitochondria are relatively less abundant in the lungs than in the submaxillary gland, but I do not think that there is any great difference between the suprarenal and the submaxillary, at least in the mouse.

Ehrlich has made a study of the oxygen saturation of organs by another and less satisfactory method. According to him the organs may be divided into three groups (quoting from Bayliss, 1915, p. 595):

"1. Those of high 'oxygen saturation,' in which indophenol blue is not reduced, such as the grey matter of the brain, the heart and some other muscular organs.

"2. Those which reduce indophenol blue, but not alizarin blue. Such are the greater number of the tissues, smooth muscle, most voluntary muscles and secreting glands.

"3. Those which reduce even alizarin blue—lungs, liver, fatty tissue, Harderian gland."

Personally, however, I have been unable to find any correspondence between the amount of mitochondrial substance and these figures of oxygen saturation.

(c) Within single cells also attempts have been made to measure oxidations. For example, R. S. Lillie (1913, p. 247) finds that "in frogs' blood-corpuscles the formation of indophenol by the intracellular oxidation of a mixture of alpha-naphthol and dimethyl-para-diamino-benzene takes place most rapidly in the immediate neighborhood of the nuclear and plasma membranes. The conditions at the surfaces of these structures are thus particularly favorable to rapid oxidations." One might expect to find condensations of mitochondria in these localities, but I have carefully examined mitochondria vitally stained with janus green in frogs' red blood-corpuscles and I have found that they are distributed more or less uniformly throughout the cytoplasm.

(d) Or we may ask whether, when we modify experimentally the rate of oxidation, there is any change in the mitochondria. Warburg (1910, p. 313) found that he could double the oxygen consumption of sea-urchin eggs by the addition of small amounts of sodium hydroxide to the sea-water. Adrenaline increases the oxygen consumption of tissues and cyanide inhibits it. Israel's (1891, p. 334) account of changes in the bioblasts of Altmann (mitochondria) in the cells of the kidney following experimental ligation of the renal artery deserves confirmation and extension. He found that they reacted very quickly—that is to say, in the course of several hours. Champy's description of the solution of mitochondria through asphyxiation in the center of pieces of tissue, remote from the surrounding oxygen, grown in serum, may have more bearing upon the question.

(e) It would be profitable also to inquire into the condition of mitochondria in intestinal worms which are anaerobic and which normally live in the absence of oxygen. *Ascaris* is a good example. According to Mathews (1905, p. 333):

“The only difference between anaerobic and aerobic respiration is that the anaerobic protoplasm is so powerful a reducing agent that it is able to drive hydrogen out of the water, thus oxidizing itself without the aid of atmospheric oxygen to act as a depolarizer. Aerobic protoplasm being less powerfully reducing, requires the presence of more or less oxygen to take care of the hydrogen. The difference between these different kinds of protoplasm is exactly the difference between metallic sodium and metallic iron.”

If this be true we would expect the mitochondria to be of unusual abundance; but Bayliss (1915, p. 611) does not seem to subscribe to it. As a matter of fact, judging by the work of Romeis (1913*a*, p. 9) and others, the mitochondria in *Ascaris* do not seem to be peculiar. Leeches can live for upwards of ten days in the absence of oxygen, yet the illustrations of Grynfeldt (1912*b*, p. 263) of their mitochondria do not seem to show any noteworthy differences from those of other annelids. The cells of the gas-bladder of fishes, which actually secrete oxygen (Woodland, 1911, p. 225), constitute another field for study.

(f) And finally, in the condition of acidosis, there is an inhibition of the respiratory oxidation. It may be induced experimentally in a variety of ways. In some preliminary experiments which I have made with rabbits by poisoning with illuminating gas I have failed to detect any alteration in the mitochondria of the lymphocytes in the circulating blood stained with janus green, and I think that one would expect to find changes in them earlier than in the other tissues.

While the hypothesis that the mitochondria are concerned in protoplasmic respiration is very attractive and meets some of the requirements, it should nevertheless be tested experimentally in many directions before it can be unreservedly accepted.

But we can hope for a more accurate analysis along purely experimental lines by altering the rate and condition of the various vital processes and noting the effect, if any, upon the mitochondria. We must bear continually in mind the difficulty of dissociating functionally between the mitochondria and their environment. A change in the mitochondria does not necessarily mean that they are concerned in the process in question, for they may be purely passive agents, the change in their appearance being entirely due to some alteration in the protoplasm

in which they are embedded—some variation in fluidity, refractive index, or electrical state, for instance. And conversely, the absence of any noticeable reaction on the part of the mitochondria does not exclude the possibility of their participation. Interpretation is extraordinarily difficult. The methods of tissue culture, if rigidly controlled, may prove of service.

The most pressing need, however, is for further knowledge of the chemistry of mitochondria, for, when chemical facts come in at one door, superstition invariably vanishes at the other. Here also the difficulties seem almost insurmountable, because we have had to rely upon indirect and roundabout methods. If it were possible to make a direct chemical analysis of mitochondria it would place the whole work, once and for all, upon a secure foundation, but doing this involves very special training and it is hard to see the way. It might be possible, as I have already suggested, to partly separate out mitochondria by means of the centrifuge and then collect them for analyses by some of the recently devised methods of cell dissection. It would seem that some such procedure might be of use in the analysis of many other cytoplasmic constituents. Should it prove feasible it would have a most illuminating effect upon the whole question of the constitution of protoplasm.

EXPLANATION OF FIGURES OF PLATE.

Figures 1, 3, 5, 6, 10, and 13 have been drawn from preparations fixed in Regaud's formalin and bichromate mixture and stained with fuchsin and methyl green. Figure 7 was drawn from material fixed in the same way but stained with iron hematoxylin. In making the drawings Zeiss apochromatic objective 1.5 mm., compensating ocular 6, and camera lucida were used giving a magnification of 1,500 diameters.

FIG. 1. Kidney-cells of white mouse.

FIG. 2. Cells of trapezoid nucleus of white mouse, remarkable for the large block-like mitochondria in the peripheral cytoplasm (after Nicholson, 1916).

FIG. 3. Vesicula seminalis of white mouse. Note the absence of blebs on the mitochondrial filaments and the stages in the production of the secretion.

FIG. 4. Large anterior horn nerve-cell of white mouse (after Nicholson, 1916).

FIG. 5. Prostate of white mouse containing very minute mitochondria.

FIG. 6. Ovarian egg of white mouse containing for the most part granular mitochondria.

FIG. 7. Serous cells of parotid of mouse with very fine rod-like mitochondria.

FIG. 8. Thyroid vesicle (after Bensley, 1916).

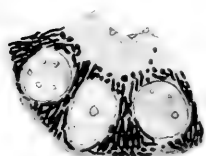
FIG. 9. Pancreas (after Scott, 1916), with typical bleb-like swellings on the mitochondrial filaments.

FIG. 10. Intestinal epithelium of white mouse with bipolar arrangement of mitochondria.

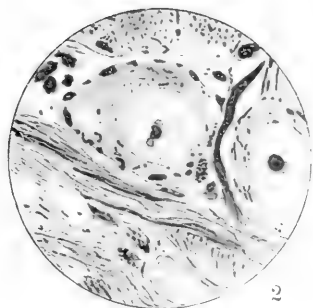
FIG. 11. Large cell of mesencephalic nucleus of the fifth nerve with small cell of locus caeruleus adjacent (after Nicholson, 1916). Note the difference in the mitochondria.

FIG. 12. Large pyramidal cell of hippocampus (after Nicholson, 1916).

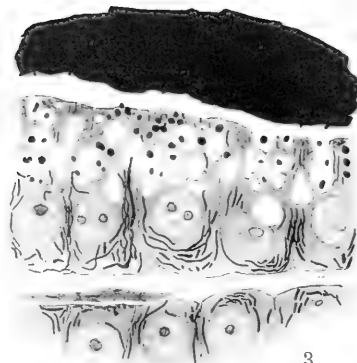
FIG. 13. Thymus of white mouse, showing large mitochondria in the small round cells, tiny mitochondria in the epithelial cells, and an apparent absence of mitochondria in the mast cell.



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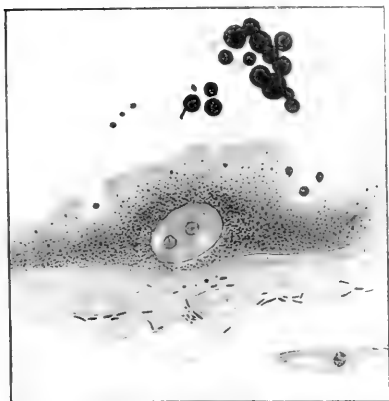
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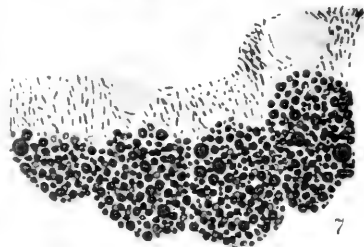
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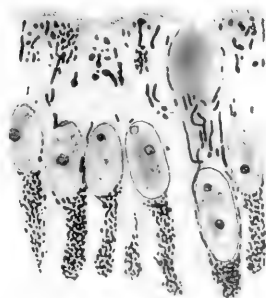
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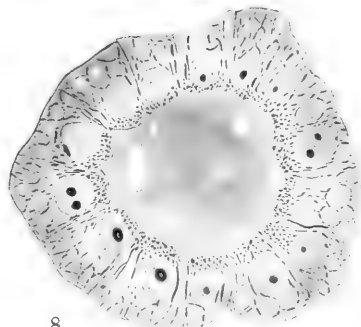
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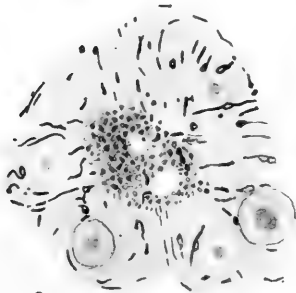
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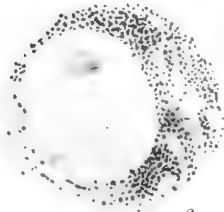
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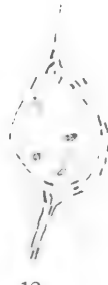
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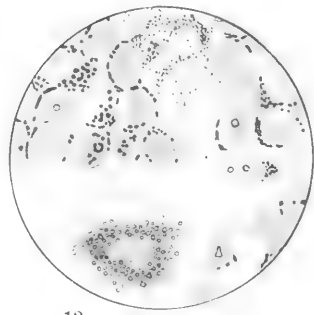
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CONTRIBUTIONS TO EMBRYOLOGY, No. 26.

THE DEVELOPMENT AND REDUCTION OF THE TAIL AND OF THE
CAUDAL END OF THE SPINAL CORD.

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Four plates, two text-figures

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THE DEVELOPMENT AND REDUCTION OF THE TAIL AND OF THE CAUDAL END OF THE SPINAL CORD.

BY KANAE KUNITOMO.

INTRODUCTION.

A great deal of literature has been published from time to time dealing with the question of whether the human embryo at a certain stage of its development has an actual tail—that is, a structure homologous with the tail of other mammals—and with the persistence of such tail in after life. In biogenetic investigation this is a subject of great interest (Darwin and Haeckel). It was from the assumption of the occurrence of a tail in the human embryo, which was based upon one of Ecker's figures (*Icones Physiologicae*, 1851-59), that Darwin drew one of his arguments for the descent of man from a race of tailed ancestors. Von Kölliker (1884) asserted that the human embryo has a tail-like process at its caudal end which was not, however, recognized by him as a "true" tail. He described it as *eine spitze Schwanzartige Verlängerung*. Ecker (1851-59) referred to it as *Schwanzformige Körperende*, and stated that it contained only the notochord and caudal end of the spinal cord, and was converted finally into a coccygeal tubercle (*Steisshöcker*) projecting caudalward. Rosenberg (1876, 1899), who investigated the subject from a morphological standpoint, opposed the theory that the caudal appendage in the human embryo was homologous with the tail of other mammals, as he could not discover any part of the axial skeleton in the caudal projection. He believed, therefore, that the latter must be concerned more with the development of the spinal cord which he found embedded in its dorsal side. Ecker (1880), on the other hand, held it to be a true tail, even though it contained no vertebral primordia, and from his conclusions on the subject interest and discussion were revived. His (1880*a*), who coincided in general with Ecker, published his theories under the heading "Besitzt der menschliche Embryo einen Schwanz?" in his *Anatomie menschlicher Embryonen*. He recognized a tail-like appendage in his younger specimen (4 mm.), but did not regard it as a true tail. In older embryos, in which the primitive vertebræ had developed into cartilaginous tissue, he found that one or two vertebræ entered into the root of the tail. This portion he designated as the *vertebral tail*. The remainder contained only notochord and medullary cord (caudal filament) and was therefore called *non-vertebral tail*. In none of his specimens did he find more than the normal number of vertebræ, 34. He states: "Die Embryonen A and B haben sonach eine ächte Schwanze-anlage, die aber ausserordentlich kurz ist und jedenfalls nicht über zwei Segmentlängen umfasst." The opinions of Ecker and His may be summarized as follows:

1. The term *tail* refers only to that portion of the embryo which projects beyond the cloaca.

2. In younger specimens (8 to 15 mm.) the tail appears as a free, pointed projection from the cloaca, directed caudo-dorsally.

3. The tail consists of two portions - that containing vertebræ and that without vertebræ. The latter contains only chorda dorsalis and medullary tube. In time this portion disappears, the medullary tube atrophying and the chorda becoming converted into a knot.

4. The vertebral portion persists for a while, appearing later as a coccygeal prominence in the caudal region—coccygeal tubercle; then it, too, disappears.

Keibel (1891) published in an important paper his findings in regard to the development of the caudal gut in 4, 8, and 11.5 mm. embryos. The existence of this structure, which forms a small canal or cell-strand at the caudal end of the body axis, he regards as irrefutable evidence of a tail primordium. He found the gut to be longer in the 8 mm. embryo than in the others. He asserts (page 378) that in this stage the caudal gut extends through the whole length of the tail, and apparently at this time attains its maximum length. This author defines the line of demarcation between the tail and the body in two ways: (1) he designates as the *tail* the caudal portion beyond the attachment of the pelvic joint; (2) in the younger embryos, in which the primordia of the legs have not yet appeared, he defines the first 8 trunk segments as the cervical segments, the next 12 as the dorsal, the next 5 as the lumbar, the next 5 as the sacral, and the remaining segments as caudal vertebræ. These he found were usually 6 in number. The last one he called the *mesodermic remnant* and regarded it as one segment, although it was two or three times as long as those cranial to it.

Braun (1882) published his observations on the development and reduction of the embryonic tail among mammalia, having at his disposal a great number of specimens. As a rule he found a caudal filament at the extreme end of the tail in the mammals that he studied, and therefore believed this structure to be of general occurrence, and probably true also of the human tail. On the other hand, Ecker and His, who studied the same condition in human embryos, did not consider the two exactly homologous. Braun classified the two portions of the tail as internal and external, and subdivided the latter into vertebral and non-vertebral tail, the caudal filament being part of the latter. Waldeyer (1896) takes exception to this division into internal and external tail, as he does not believe the former is a tail. Rodenäcker (1898) uses the terms *cauda aperta* and *cauda occulta* instead of internal and external tail. Unger and Brugseh (1903) give the following results of their investigations:

1. In the reduction of the tail the caudal vertebræ fuse to form the last vertebra.
2. The caudal filament represents the remnant of the tail-bud and contains a branch of the middle sacral artery.
3. In the reduction of the tail two processes are concerned: (*a*) the formation of the caudal tubercle; (*b*) the formation of the coccygeal tubercle. The first is the reduced tail; the second is formed by the bulging of the caudal end of the vertebral column. This is due to the fact that the growth of the vertebræ is more rapid than that of the skin and spinal cord.
4. The connective tissue contained in the caudal filament develops into the caudal ligament.

Regarding these changes they state (p. 100):

“Wandelt sich dann durch stärkeres Wachstum der Kaudalwirbelsäule der Schwanzhöcker in den Steisshöcker um, so wird durch den Zug der Haut, deren Wachstumsrichtung der der Steisswirbel entgegengesetzt ist, der Schwanzfaden von der Achse der Kaudalwirbel entfernt und mit der Haut aufwärts mitgenommen (cfr. Embryo Dü. 4 $\frac{1}{2}$ em.). Durch dieses Aufwärtsrücken des Schwanzfadens wird aber dieser seines Rückenmarks beraubt, d. h. er ist reduziert. Seinen Inhalt stellt nun ein Gewebe vor, das in Form von Bindegewebszügen mit der kaudalen Fläche des letzten Kaudalwirbels verbunden ist, und das aus dem Mesodermrest des früheren Schwanzfadens hervorgegangen ist. Da auch hier diesen Bindegewebszügen und dem Schwanzfadenrest die Endäste der inzwischen durch Bildung des Steisshöckers sehr reduzierten Arteria sacralis media zukommen, so können wir sie als einen immerhin wesentlichen Rest der ursprünglichen Schwanzanlage bezeichnen. Diese Bindegewebszüge sind das lig. caudale; sie schliessen auch den ursprünglich im Schwanzfaden sich befindlichen kaudalsten Teil des Rückenmarks ein, in dem sich später die ‘vestigis coccygiens’ von Tourneux und Hermann (s. o.) oder ‘kaudalen Rückenmarksreste’ entwickeln.”

Our knowledge concerning the development of the caudal end of the spinal cord is very limited, especially as regards its early stages. My aim, therefore, has been to study the early development of this part of the spinal structure and to follow the histological changes it undergoes in adaptation to later topographical conditions. Before reporting my investigations, however, I would refer to some of the writers who have preceded me in this field of study. Clarke (1859), in his well-known study of the spinal cord, pictures the ventriculus terminalis as seen in sections of the cord of the ox (plate XXIII, fig. 21). He regarded this structure as a persisting remnant of the lower end of the sinus rhomboidalis, which in other mammals is usually limited to the lumbar enlargement. Krause (1875) discovered the ventriculus in the spinal cord of the human embryo and describes it as persisting in adults as a rudimentary organ. He suggests that in the embryo, by means of its ciliated cells, it serves in the maintenance of the circulation of the contained cerebral spinal fluid. Tourneux and Hermann (1887), who studied the caudal end of the spinal cord in the human embryo, discovered the remnant of the neural canal in the caudal region and called it *vestigis médullaires coccygiens*. They describe in detail the process of reduction of the caudal end of the spinal cord. Argutinsky (1898) discussed the morphology of the ventriculus terminalis in older fetuses and newborns, and classified it in three divisions—upper, middle, and lower. Von Kölliker, Ecker, His, and others reported that in younger embryos the spinal cord extends to the extreme end of the tail. Brugsch and Unger briefly summarized their investigations on the ventriculus terminalis in the human embryo as follows (p. 232):

“Kurz gesagt stellt der V. t. also eine konische Erweiterung des Centralkanals im unteren Ende des Conus medullaris und im Anfange des filum terminale vor, dessen oberer weiter Abschnitt meistens Ausbuchtungen besitzt. Der untere Abschnitt endigt blind im filum terminale.”

In describing this structure they make the following divisions: (1) an upper, wider part, which is continuous with the central canal of the more cephalic part

of the spinal cord, and which forms an irregular, evaginated space in the conus medullaris; (2) an under part, which gradually narrows toward its caudal end and terminates blindly in the filum terminale.

The various investigations of the occurrence of tails among adults, children, and newborn infants have given rise to a great deal of discussion. Bartels (1884) published an exhaustive study of the occurrence of tails among the human race. Other publications on the subject have appeared from time to time, notably by Virchow (1884), Oskar Schaeffer (1892), Pyatnitski (1892), Dickinson (1894), Berry (1894), Kohlbrügge (1898), Watson (1900), and others. Harrison (1901) describes the histological structure of a large, well-developed tail which was removed from a child six months old. He states:

“Two weeks after the birth of the child the tail was 4.4 cm. long; at the age of two months it had grown to 5 cm., and at six months, when it was removed, it had attained a length of 7 cm., showing altogether a fairly rapid rate of growth. The most remarkable characteristic of the tail was its movability. * * * Beneath the skin the main bulk of the tail was made up of areolar tissue containing much fat. Blood vessels, nerves, and striated muscle fibers are embedded in this mass. There is no trace of anything like the medullary cord or of notochordal tissue.”

More recently similar observations have been made by Brugsch (1907), Konstantiowitsch (1907), and Schwarz (1912).

MATERIAL AND METHODS.

The material upon which this study is based consists of 44 specimens in the Carnegie Collection of human embryos, Baltimore. They range from 4 to 125 mm. C.R. length, and a table of them, with their respective measurements, is shown herein. The specimens, for the greater part, had been carefully preserved in 10 per cent formalin and dehydrated in alcohol. The smaller ones were embedded in paraffin, the larger ones in celloidin, and most of them were cut in serial sagittal sections varying in thickness from 20 to 200 μ in the different specimens. A large proportion of the specimens were stained *in toto* in alum cochineal and borax carmine before embedding; others were stained on the slides with hematoxylin and eosin or similar stains. From 15 to 80 sagittal sections through the median part of each embryo were used in this investigation, and usually one graphic reconstruction was made of each specimen, although all of these are not illustrated. They present a median profile view disclosing some structure—for instance, the winding caudal end of the chorda dorsalis or the sympathetic ganglia. In the illustrations these are slightly schematicized in order to show distinctly their actual relations. The graphic reconstructions were made by copying each section on transparent paper from a projection apparatus. When the drawings under the projection apparatus were completed the sheets were piled so that adjacent sections were accurately fitted one upon another. The desired parts of the sketch on each sheet were then copied on another sheet of paper, due attention being given to the form and relation of the component parts. This procedure was facilitated by the use of a glass table illuminated from below. In the case of cross-

sections, a guide-line was established by marking upon each sheet two lines, one perpendicular to the other, thus forming a series of crosses which were exactly superimposed throughout the entire pile. The individual sections were then plotted off on millimeter paper by fitting the crosses to a chosen perpendicular line, the distance between the sections being determined by the thickness of the sections and the enlargement of the drawing. The enlargements with the projection apparatus were as follows: Embryos 4 to 16 mm., $\times 70$; embryos 17 to 38 mm., $\times 50$; embryos 39 to 52 mm., $\times 30$; embryos 67 to 125 mm., $\times 20$.

Table of Embryos Studied.

Length (CR) of embryos	Catalogue No.	No. of somites.	No. of spinal ganglia.	Segmental level of cloaca.	Extension of caudal end of spinal cord.	Level of demarcation between main cord and its more caudal atrophic portion.
mm.						
4.0	836	28+remnant.				
4.0	786	30+remnant.				
5.5	810	L. 37+remnant. R. 36+remnant.	32	29th segment	Tip of tail	
6.6	371	37+remnant.	32	29th segment	Do.	
7.5	221	38+remnant.	31	30th segment	Do.	
8.0	389	38+remnant.	32	Between 30th and 31st segments.	Do.	
9.0	422	38+remnant.	32		Do.	
9.0	721	37	32			
10.0	1197	35+remnant.	32	Between 31st and 32d segments.	Tip of tail	
11.0	544	38	32	33d segment.	Do.	
12.0	852	37	33	Between 32d and 33d segments.	Do.	33d segment.
13.0	485	37	33		Do.	33d segment.
13.0	643	37	33	32d segment.	Do.	(?)
14.0	940	36	33			33d segment.
15.5	390	35	32	Between 33d and 34th segments.	Nearly to tip of tail.	32d segment.
16.0	406	36	31		Do.	Between 33d and 34th.
16.0	43	37	32	Between 34th and 35th segments.	Do.	34th segment.
17.0	576	35	31	33d segment.	Do.	32d segment.
17.0	991	34	31	33d segment.		32d and 33d segments.
18.0	432	34	32	33d segment.	Tip of tail.	31st segment.
19.0	431	33	32	33d segment.	Do.	32d segment.
21.0	837	35	32	33d segment.	Do.	32d segment.
23.0	453	35	32	35th segment		31st segment.
23.0	382	34	31	Below last segment	Nearly to tip of tail.	Between 31st and 32d.
24.0	632	35	31	35th segment	Do.	Between 32d and 33d.
25.0	584a	34	32	34th segment	Do.	32d segment.
26.0	1008	34	31	34th segment		Between 31st and 32d.
26.0	405	34	31	34th segment	Tip of tail	Between 30th and 31st.
27.0	875	34	31	34th segment.	Nearly to tip of tail.	Between 31st and 32d.
30.0	75	34	31		Tip of tail	32d segment.
33.0	145	35	31	34th segment		Between 30th and 31st.
33.0	211	34	31	34th segment	Do.	Between 30th and 31st.
35.0	199	34	30	Below last segment.	Do.	31st segment. (?)
36.0	449	32	31	Do.	Below last vertebra	Between 29th and 30th
37.0	972	34	30	Do.	Do.	31st segment.
39.0	362	34	30		Do.	Between 29th and 30th.
46.0	95	35	30			(?)
50.0	184	34	R. 30 L. 29			Between 29th and 30th segments.
50.0	96	35	29			
52.0	448	34	30			
67.0	1656	34	29			
80.0	662					
100.0	928					
125.0	142					

SERIAL DESCRIPTION OF EMBRYOS.

EMBRYO No. 836, 4 MM. GREATEST LENGTH.

This embryo represents the earliest stage studied in this investigation, and a diagrammatic profile reconstruction of it is shown in figure 1, plate 1. It was sectioned transversely and a series of models of it were reconstructed under the direction of Professor Evans. These were available for comparison and were of particular value in orienting the sections in the lumbo-sacral region, where the caudal extremity curves so that it lies transverse to the axis of the trunk. The embryo contains 28 somites and a long mesodermic remnant which extends about one-third of its length beyond the caudal end of the spinal cord. The chorda dorsalis runs along the ventral surface of the spinal cord in close apposition to it and terminates before reaching the caudal end of the central canal; whereas the caudal gut extends farther down towards the tip of the tail, as indicated in figure 1. The caudal extremity of the embryo consists of a mass of germinating cells into which the ends of the spinal cord, the chorda dorsalis, and mesodermic remnant all merge, their outlines becoming entirely obliterated. The primordium of the caudal artery extends to the tip of the tail.

EMBRYO No. 786, 4 MM. GREATEST LENGTH.

This specimen has 30 somites and a mesodermic remnant; the latter consists of a long cord joined to 4 globular masses, the last of which is slightly longer and thinner than the others. The counting of the somites in small embryos is always very difficult, as pointed out by Keibel, especially when the material is not well preserved. It was easy, however, to make out the vesicula auditiva in the sagittal sections of this specimen, and caudo-dorsal to it the glossopharyngeal nerve. The ganglionated vagus nerve in turn is situated caudal to this, being surrounded by the internal jugular vein, which curves around its caudal margin. The first cervical somite lies dorsal-caudal at a distance of 180 to 200 μ from the vagus ganglion. The embryo has 3 occipital somites.

EMBRYO No. 810, 5.5 MM. CROWN-RUMP LENGTH.

A graphic reconstruction of the caudal end of embryo No. 810 is shown in figure 2, plate 1, in which the structures are diagrammatically straightened out. In reality the caudal end is bent to the right and towards the front, its tip nearly reaching the right side of the face. The first cervical ganglion is about one-third the size of the second and lies close to the Frieriep ganglion, which is situated at the dorsal side of the bow-shaped trunk of the accessory nerve and is particularly large on the left side. There are 32 spinal ganglia, the last 2 being small. I was able to count on the right side 37 somites and a mesodermic remnant; on the left side only 36 somites and a remnant. In the cranial portion of the mesodermic remnant there seem to exist potential somites, the outlines of which, however, can not yet be made out. Its caudal part consists of a small, bell-shaped remnant. The entire remnant is about the length and size of 4 somites. The mesoderm in the caudal end of the embryo is primitive in character and can be seen dividing into its parietal and visceral layers. Embedded in these can also be seen the caudal gut and a simple vascular plexus. The spinal cord, with its central canal, extends to the caudal end of the mesodermic remnant. The caudal gut, which extends from the cloaca to the end of the tail, is beginning to disappear in this embryo at a point 200 μ caudal to the cloaca and 657 μ from the extreme end of the tail between the thirtieth and thirty-first somites, as is indicated in figure 2, plate 1. In figure 31, plate 2, these conditions are shown more in detail. At the site of the beginning degeneration the ends are sharply pointed, but are still connected by a cell-strand. The shorter, cranial portion of the caudal gut opens into the cavity of the cloaca. The longer, caudal portion ends blindly at both extremities, the caudal end merging into the mesodermic cell-mass and uniting with the caudal end of

the chorda dorsalis. Each portion of the gut contains a distinct lumen. On the ventral surface of the caudal region of the embryo, where the gut first begins to disappear, is a small groove (indicated by \times in fig. 31), which may represent the primordium of the sub-caudal epithelial plate of Keibel.

EMBRYO No. 371, 6.6 MM. CROWN-RUMP LENGTH.

This embryo has 37 somites and a mesodermic remnant. The thirty-seventh somite lies close to the remnant, while the others are separated by narrow spaces into which blood capillaries enter. The remnant is constricted at three points, the separations, however, being incomplete. The caudal end of the medullary tube extends to the end of the tail, as can be seen in figures 3 and 32. The caudal gut, which was distinctly recognized in the 5.5 mm. specimen, has here undergone a more marked obliteration, and over its greater part is left only a strand of cells which, to judge by their staining reactions, are probably degenerating. The caudal portion of the gut contains a small lumen, however, and shows no change from that noted in the preceding specimen. What constitutes the cranial end of the shorter portion of the caudal gut in the younger embryo is here dilated and forms part of the cloacal membrane. Ventral and parallel to the gut is a blood-vessel which anastomoses with the middle sacral artery by means of numerous capillaries. The chorda dorsalis runs within the substance of the vertebral column until it reaches the thirtieth somite, when it emerges from the vertebral tissue and continues the remainder of its course in the interval between the primitive vertebrae and spinal cord, until it finally loses itself in the cell-mass at the end of the tail. The plexiform middle sacral artery follows a course ventral to the vertebral column and can be traced to the tip of the cord.

EMBRYO No. 221, 7.5 MM. CROWN-RUMP LENGTH.

Embryo No. 221 has 38 somites and a remnant, the latter showing constrictions at three points. The caudal end of the embryo resembles that of a pig, as described by Keibel in his work on the human embryo. The cloaca, which is situated at a level with the thirty-first somite, is well developed, but as yet there has been no perforation of the membrane. Below the chorda dorsalis is a short remnant of the caudal gut containing a small lumen, as can be seen in figure 4, plate 1. At their caudal extremities the chorda, spinal cord, and caudal gut appear to fuse together. The central canal of the spinal cord is obliterated at its sacral portion, showing a pathological condition, and appears as a solid mass of nervous tissue. The groove between the tail-bud and the cloaca, which in the younger specimens indicated the first point of disappearance of the caudal gut, is here situated at the level of the thirty-first somite and is destined to later develop into the sub-caudal epithelial plate. In this embryo there are 31 spinal ganglia with nerves.

EMBRYO No. 389, 8 MM. CROWN-RUMP LENGTH.

As can be seen in figures 5 and 33, the caudal gut in embryo No. 389 still persists as a group of cells inclosing a narrow lumen. This mass seems to fuse with the caudal ends of the medullary tube and the chorda. The remnant of the caudal gut is surrounded by a close network of capillaries, which possibly bear some relation to its absorption. At the ventral wall of the spinal cord below the thirtieth somite can be seen two or three folds indicated by \times in figure 33. As such folds were not found in the younger specimens, I assume that they begin at about this stage of development, and from now on I shall have occasion to refer to them frequently. The winding chorda emerges from the vertebral column at the thirty-fourth somite and continues its course to the end of the tail in the interval between the vertebral column and the spinal cord. This embryo contains 38 somites and a mesodermic remnant. In the latter I was unable to make out any constrictions, only a mass of germinating cells. The spinal ganglia number 32.

EMBRYOS No. 721 AND No. 422, 9 MM. CROWN-RUMP LENGTH.

As no embryos of this size in sagittal section were available, I have had to make use of two that were cut in coronal sections, although in them the study of the vertebral structures was much more difficult. In embryo No. 721 the caudal portion is cut transversely, and in the sections through the end of the tail the caudal gut with its round lumen can be distinctly recognized. The extreme end of the gut is surrounded by a network of capillaries communicating with the middle sacral artery and vein. The cells of the caudal gut seem to fuse with those of the spinal cord and the chorda dorsalis in the caudal region. In this specimen there are 37 somites and a long remnant. I was able to count 32 spinal ganglia, the thirty-second being small and without nerves. In embryo No. 422 there are 38 somites with a remnant, and 32 spinal ganglia. The last one of these also is small and contains no nerve-fibers. The specimen is so poorly preserved that the caudal gut can not be clearly made out.

EMBRYO No. 1197, 10 MM. CROWN-RUMP LENGTH.

In embryo No. 1197 there are 35 primitive vertebræ of Remak, or scleromeres, although the thirty-third and thirty-fourth appear to consist each of two parts fused together. This fusion has occurred, apparently, at an earlier stage. At the caudal end of the vertebral column the vertebræ have not completely developed, although the tissue is condensed, showing that development is well under way. The chorda dorsalis is situated dorsal to the primitive vertebræ in the caudal portion and is slightly contorted. Caudally it terminates suddenly with a rounded end ventral to the neural tube at a level with the thirty-fifth vertebra. There are 32 spinal ganglia with nerves, the last 2 nerves being quite delicate. I have carefully examined each section of the caudal region in an effort to locate a remnant of the caudal gut, but could find no trace of it.

EMBRYO No. 544, 11 MM. CROWN-RUMP LENGTH.

In embryo No. 544 the caudal end is bent sharply to the right. In the graphic reconstruction shown in figure 34 it is represented as straightened out in order to show more clearly the relations of the structures in this region. It is pictured in a more diagrammatic way in figure 6. Here 38 primitive vertebræ and a remnant are present, the latter differing from that described in the younger embryos. In those instances I have referred to it as the mesodermic remnant, using the terminology of Keibel, whereas in this stage of development (9, 10, and 11 mm.) the mesodermic remnant has been gradually converted into a non-vertebrated tail. The last scleromere or primitive vertebra, as shown in figure 34, is larger than the two or three more cranially situated ones. Two theories as to its development present themselves for consideration: Its growth may be the result of (1) fusion of the last two or three scleromeres which have become separated from the adjacent somites; (2) the addition of cells from the mesodermic remnant. While somewhat in doubt as to which theory to accept, I am inclined to favor the latter, as this embryo contains 38 primitive vertebræ, corresponding to the maximum number of somites found in the younger embryos, and there is no condensed tissue or group of cells in the end of the tail to indicate the primordium of a primitive vertebra.

EMBRYO No. 852, 12 MM. CROWN-RUMP LENGTH.

In embryo No. 852 I found 37 scleromeres and a remnant; also 33 spinal ganglia, the last 2 being small, with slender nerves. The last 3 nerves emerge at the same point to form the caudal nerves, which run from about the thirty-second to the thirty-sixth scleromere. The central canal of the spinal cord narrows between the thirty-fourth and thirty-fifth scleromeres and on the ventral wall of this narrow part are a few folds. These are indicated in figure 35. The chorda is almost entirely embedded in the tissue of the primi-

tive vertebræ. In the younger specimens it emerges at the thirty-fifth scleromere, while in this one it emerges at about the thirty-seventh and from thence runs ventral to the spinal cord, touching the latter closely. The cloaca is situated at a level between the thirty-third and thirty-fourth scleromeres.

EMBRYO No. 485, 13 MM. CROWN-RUMP LENGTH.

This specimen is cut in transverse section and is therefore well suited for the study of the spinal cord. There are 33 spinal ganglia, but at the thirty-third and thirty-second complete nerve-fibers can not be made out. The thirty-third, in particular, comprises such a small cell-group as to be hardly recognizable. There are 37 primitive vertebræ and a non-vertebrated tail portion 289 μ long. As in the several specimens immediately preceding it, the few caudal vertebræ are fused together, showing no distinct boundaries. The non-vertebrated tail portion consists still of germinating mesenchymal cells, while the more cranially situated scleromeres are gradually becoming converted into precartilaginous tissue.

EMBRYO No. 643, 13 MM. CROWN-RUMP LENGTH.

This specimen is cut in serial sagittal sections, but is, however, rather poorly preserved. There are 37 primitive vertebræ, the last one being incomplete. Caudal to this is a long non-vertebrated tail portion. There are 33 spinal ganglia, the last 2 having slender nerves which, with the thirty-first, form the caudal nerves. These extend caudally along each side of the tail.

EMBRYO No. 940, 14 MM. CROWN-RUMP LENGTH.

This specimen is cut in transverse section, and it is therefore difficult to follow the topography of some of the structures. I was able to count 36 primitive vertebræ, with a remnant, and 33 spinal ganglia. The last two, especially the thirty-third, are small and not provided with nerves. The twenty-ninth, thirtieth, and thirty-first spinal nerves extend down along the sides of the cord to the level of the thirty-sixth vertebra. The central canal of the spinal cord narrows at about the thirty-second vertebra, the portion caudal to this being practically devoid of the mantle layer.

EMBRYO No. 390, 15.5 MM. CROWN-RUMP LENGTH.

In embryo No. 390 the primitive vertebræ are differentiated into precartilaginous tissue and between the vertebræ there is a small quantity of embryonic connective-tissue, as indicated in figure 36. At this period the vertebral column consists of 35 precartilaginous vertebræ; no additional segments can be recognized, although in the younger specimens there were 38 somites and one very long mesodermic remnant. There is a long, irregular, mesodermic cell-strand at the caudal end of the thirty-fifth segment, which, with the spinal cord, extends to the end of the tail. Between the two lies the caudal end of the chorda dorsalis, which, however, does not extend to the tip of the tail, as can be seen in figures 8 and 36. About the thirty-fifth segment the chorda becomes embedded in the substance of the vertebral column, the point at which it emerges being characterized by a sharp curve in its course.

We are here confronted with the following questions: What was the fate of the additional somites which could be so distinctly recognized in the earlier stages? And what is the mesodermic cell-strand which extends from the last primitive vertebra? As to the first, from the study of this material I am of the opinion that the last few vertebræ, which have earlier developed from the sclerotomes, fuse together during the process of embryonic development, thus forming the last vertebra in an embryo of this age. I am, however, aware that the comparison of a series of embryos can not conclusively settle this question. As concerns the cell-strand, it is my belief that it is formed of parts of the tissue which did

not go to make up the primitive vertebrae, and constitutes the primordium of the caudal ligament. As is well known, the sclerotomes of the somites develop into not only primitive vertebrae, but also into several other supporting tissues which form the framework of the vertebral column. (Keibel and Mall, *Human Embryology*, I, page 331.)

The spinal cord narrows suddenly at the thirty-second vertebra, so that the portion caudal to this, together with its canal, presents an appearance distinctly different from the main body of the cord, as can be seen in figure 36. On account of its regressive appearance, I shall hereafter refer to this part as the atrophic cord. One of its very characteristic features is a slender, narrow canal, and it might be desirable to speak of this as the narrow canal portion of the spinal cord.

EMBRYO No. 406, 16 MM. CROWN-RUMP LENGTH.

A graphic reconstruction of embryo No. 406 is shown in figure 37, and a more diagrammatic sketch in figure 9. These show that the embryo has 36 cartilaginous vertebrae. On the right side the thirty-second and thirty-third segments have fused together. The last vertebra consists of two or three sections, each of which in an earlier stage probably represented a complete somite, these later fusing into one large segment. At the caudal end of this is a group of undifferentiated mesodermal cells—the primordium of the caudal ligament. The caudal end of the chorda dorsalis emerges at the thirty-sixth vertebra and terminates abruptly between the vertebral column and the spinal cord. The spinal cord narrows suddenly at the thirty-fourth vertebra. On the ventral wall of the canal in this narrow or atrophic portion of the cord there are three or four folds (fig. 37). In some of the sections can be seen a larger fold at the level of the twenty-ninth vertebra, which hangs down to the level of the thirty-fourth, both sides adhering to the wall of the spinal cord, thus forming a diverticulum, which is not shown in the illustration. The caudal end of the embryo is bent sharply dorsal, the bent portion being marked off on the surface by a shallow, circular furrow. The spinal cord extends to the end of the tail, conforming to the shape of the bent portion. The extreme end contains a narrow cavity which represents the caudal end of the central canal; the canal is interrupted at the root of the tail, where the cord appears to consist of solid nerve-tissue, as is indicated in figure 37. In this specimen there are 31 spinal ganglia with distinct nerves.

EMBRYO No. 43, 16 MM. CROWN-RUMP LENGTH.

This specimen has 37 cartilaginous vertebrae, the last being divided into three parts. There are 32 spinal ganglia. A graphic reconstruction was made of this embryo, but it is not illustrated in the figures.

EMBRYO No. 576, 17 MM. CROWN-RUMP LENGTH.

This embryo has 35 cartilaginous vertebrae, the last consisting of three small pieces fused together. The demarcation between these pieces can be more clearly recognized in the lateral portions of the column than in the median plane; so in determining the composition of the last segment one must study carefully the more lateral line of sections. A profile reconstruction of the embryo is shown in figure 38, and a more diagrammatic sketch in figure 10. The tail, with the caudal end of the spinal cord, is bent sharply dorsalward. The spinal cord narrows suddenly at the thirty-second vertebra and from this point down the central canal, which extends the entire length of the cord, becomes much smaller and rounder, while in the more cranial portion a transverse section of it would form an elongated oval. The ventral wall of the canal in the atrophic portion presents several transverse folds, as seems to be usually the case at this stage of reduction. The caudal portion of the chorda dorsalis is convoluted and its end sharply retracted.

EMBRYO No. 991, 17 MM. CROWN-RUMP LENGTH.

The caudal end of embryo No. 991 is somewhat torn, but I feel reasonably sure that it did not exhibit a long caudal process. At a level between the thirty-second and thirty-third vertebræ the central canal of the spinal cord narrows suddenly, and on the ventral wall of the atrophic portion of the cord are two folds. There are only 31 spinal ganglia, the first cervical on each side being absent. The others are completely developed, and even the thirty-first has its full complement of nerve-fibers. In embryo No. 576, just described, this nerve was quite slender. The chorda dorsalis runs in a straight line through the cartilaginous vertebral column and emerges from the thirty-fourth vertebra without winding. This is the first specimen of the series that lacks the non-vertebrated tail. At the point where the tail is found in younger embryos this has a small projection resembling a tail-bud more than an actual tail. Between the last vertebra and this caudal projection is a mesodermic cell-mass into which enter the plexiform branches of the middle sacral artery and vein.

EMBRYO No. 432, 18 MM. CROWN-RUMP LENGTH.

Embryo No. 432 contains 34 cartilaginous vertebræ. The last is larger than the thirty-third and on its lateral side shows three divisions, each consisting of young precartilaginous tissue. The middle part, where the segments are partially fused, is made up of cartilaginous tissue, and here the vertebra is incompletely divided into two segments—cranial and caudal. A little to one side of the median line, therefore, it is possible to count 35, and more laterally, 36 vertebræ. If the scleromeres of the several somites fuse together and develop into the last cartilaginous vertebra, we may assume that the remaining mesenchymal somites left in the caudal portion of the tail form the non-vertebrated tail, and that in a more advanced stage of embryonic growth this substance develops into the caudal ligament. The chorda dorsalis shows two branches at its caudal end; one is formed at the thirty-second, the other at the thirty-fourth vertebra, and both follow a dorsal course. The more caudal branch is the longer, and its pointed extremity, which represents the caudal end of the chorda, adheres to the ventral wall of the atrophic portion of the spinal cord, as shown in figure 39. The wall of the spinal cord is quite thick in this region and at its upper portion are several folds. This condition is very interesting on account of its possible mechanical relation to the chorda, because as the caudal end of the chorda retracts it would tend to draw up the end of the spinal cord that is in the tail.

EMBRYO No. 431, 19 MM. CROWN-RUMP LENGTH.

Embryo No. 431 has 33 cartilaginous vertebræ. The last is larger than the thirty-second and consists of two segments. It would seem most probable, therefore, that it has been developed by the fusion of two or more parts. The chorda dorsalis is considerably distorted in the thirty-third vertebra, thus indicating a fusion of several primitive vertebræ, and its caudal end adheres to the ventral wall of the spinal cord (fig. 13). The spinal cord extends to the tip of the tail, and a short distance from its extremity the ventral wall shows several folds. It appears possible that with the retraction of the chorda dorsalis the neural tube is also drawn up, thus producing folds on its ventral wall. The caudal end of the anterior spinal artery winds through these folds. The primordium of the ventriculus terminalis, between the wide and narrow parts of the central canal, is seen at the level of the thirty-second vertebra. This embryo has 32 spinal ganglia, the thirty-second being incomplete and without nerve-fibers; in all the others, however, the spinal nerves are complete.

EMBRYO No. 837, 21 MM. CROWN-RUMP LENGTH.

Embryo No. 837 has 35 cartilaginous vertebræ, but the last is very small and its transition into cartilage is just beginning. It is surrounded by a voluminous mass of precartilaginous tissue resulting from the fusion of the last few scleromeres. The caudal

end of the chorda dorsalis projects from the extreme end of the vertebral column into the region of the non-vertebrated tail and appears as if previously it may have adhered to the wall of the caudal end of the neural tube. In the thirty-fourth vertebra, and between the thirty-fourth and thirty-third, the chorda shows a typical loop formation, while in its main portion it is almost straight and is situated in the midline of the column. The central canal of the spinal cord narrows sharply at the level of the thirty-second vertebra. Its ventral wall shows a few folds in the region of the atrophic portion of the cord. The spinal cord reaches to the tip of the tail and has a continuous canal throughout. The middle sacral artery and vein extend into the non-vertebrated portion of the tail.

EMBRYO No. 453, 23 MM. CROWN-RUMP LENGTH.

In embryo No. 453 there are 35 vertebrae, the last consisting of precartilaginous tissue which has not as yet developed into true cartilage. The chorda dorsalis is straight and runs through the vertebral column in the midline. Its caudal end, however, shows 4 coils, as shown in figure 40, representing a profile reconstruction of the specimen. The neural canal narrows at the thirty-first vertebra. At the level of the thirty-third vertebra a sac-shaped cell-mass lies between the spinal cord and the vertebral column, separated, however, from the wall of the former (fig. 40, diverticulum). This sac seems to have resulted from a diverticulum of the ventral wall of the neural canal, the stalk of which has been obliterated. The ventral wall of the atrophic portion of the spinal cord shows small folds at the level of the thirty-fourth and thirty-fifth vertebrae. The caudal end of the spinal cord expands slightly and its extreme end enters into the tail, which is now quite reduced. The middle sacral artery communicates with the anterior spinal artery by means of a branch that curves about the tip of the vertebral column. The subcaudal epidermal plate has nearly disappeared, while the post-anal swelling and the coccygeal tubercle have become visible. There is a shallow furrow between the tail-end and the primordium of the coccygeal tubercle, constituting a boundary between them. This embryo has 32 spinal ganglia with nerves.

EMBRYO No. 382, 23 MM. CROWN-RUMP LENGTH.

Embryo No. 382 has 34 cartilaginous vertebrae. The last three do not lie exactly in a row in the median line, as can be seen in figures 15 and 41. The ventral portions of the thirty-second and thirty-fourth vertebrae, and the dorsal portion of the thirty-third, have become converted into cartilage, whereas the remainder of these two vertebrae still consists of precartilaginous tissue, as in younger specimens. At the thirty-third vertebra the chorda dorsalis gives off a short branch in a dorsal direction. Caudal to this the chorda winds and finally disappears in the caudo-dorsal portion of the thirty-fourth vertebra. Opposite the end of the chorda the wall of the spinal cord is so thickened as to give the impression that the two might have been attached. It is to be regretted that in most of the specimens the caudal end of the chorda dorsalis is torn. At the caudal end of the last vertebra the middle sacral artery anastomoses with the anterior spinal artery through a branch, similar to that mentioned in the last specimen. At the caudal end of the embryo there is a bud-like structure of the skin. This proves to be a stunted tail, for at its root can be recognized the sharp caudal end of the spinal cord and the terminal branches of the middle sacral artery and vein. The central canal of the spinal cord narrows at the thirty-second vertebra, but the ventral wall of its atrophic portion does not exhibit the folding that usually occurs in this region.

EMBRYO No. 632, 24 MM. CROWN-RUMP LENGTH.

Embryo No. 632 is quite similar to No. 382, just described, except that it has no tail-bud. There are 35 vertebrae and 31 spinal ganglia. The caudal end of the spinal cord is represented by a strand of loosely arranged cells which extends to the epidermis at a point corresponding approximately to the root of the tail in a younger embryo, as can be seen in figure 16.

EMBRYO No. 584a, 25 MM. CROWN-RUMP LENGTH.

A profile reconstruction of the caudal end of embryo No. 584a is shown in figure 42 and a simplified sketch is shown in figure 17. There are 34 vertebræ, the last being larger than the thirty-third. Around the cartilaginous mid-portion of the last vertebra there is considerable precartilaginous tissue, which has been formed by the fusion of several scleromeres. At the thirty-first and thirty-second vertebræ the column curves ventrally. The chorda dorsalis makes a loop in the thirty-fourth vertebra and gives off short branches. The central canal narrows sharply at the level of the thirty-second vertebra, but expands again at the dorsal portion of the thirty-third and thirty-fourth. This atrophic portion, however, is not the primordium of the ventriculus terminalis. Unger and Brugsch compare it with the sinus terminalis found in the amphibian embryo. It is my belief that it represents the primordium of the coccygeal medullary vestige. The extremity of the atrophic portion extends into the tip of the tail and is provided with a lumen throughout. The caudal end of the chorda appears to have adhered to the ventral wall of the spinal cord. There are 32 spinal ganglia, the thirty-second having slender nerves. The middle sacral artery and vein enter into the tail, anastomosing through branches with the anterior spinal artery.

EMBRYO No. 405, 26 MM. CROWN-RUMP LENGTH.

Embryo No 405 has 34 vertebræ, as indicated in figure 18. The last one inclines dorsally from the axis of the vertebral column. Between the thirtieth and thirty-first vertebræ the axis of the ventral column shows a decided angle and below this point bends ventrally, presenting the coccygeal curve which is characteristic of the adult. The chorda dorsalis presents a spindle-shaped swelling at each intervertebral space and its caudal end shows a loop-formation in the thirty-third vertebra. The spinal cord narrows at a level between the thirtieth and thirty-first vertebræ; the atrophic portion, with its narrow canal, is spiral at its caudal end and enters into the blunt tail-bud. On the dorsal surface of this spiral part of the cord the epidermis is lacking; whether this is due to mechanical injury or is a natural phenomenon could not be determined. From the ventral side two branches of the anterior spinal artery enter. This embryo has 31 spinal ganglia completely supplied with nerves. The middle sacral artery anastomoses through a branch with the anterior spinal artery.

EMBRYO No. 1008, 26 MM. CROWN-RUMP LENGTH.

Embryo No. 1008 has 34 vertebræ, the last being larger than any of the others and divided incompletely into two segments, as diagrammatically shown in figure 19. The end of the chorda dorsalis presents a number of intricate coils and its caudal extremity lies against the thick wall of the spinal cord. The spinal cord narrows at the thirty-second vertebra, thus marking a boundary between the atrophic portion and the upper part of the cord. The walls of the atrophic portion show two folds—one on the ventral, the other on the dorsal wall. The former lies in the region of the thirty-fourth vertebra and is similar to those already seen. The one on the dorsal wall, at a level between the thirty-second and thirty-third vertebræ, is a diverticulum which projects caudo-dorsally and contains a long, slender cavity continuous with the central canal. Such folds or diverticula are seldom seen on the dorsal wall of the caudal end of the spinal cord. In the other specimens studied folds were frequently encountered at this end of the cord, but always on the ventral wall. On the ventral wall of the upper, wider part of the canal, at a level between the twenty-ninth and thirtieth vertebræ, is another and much larger fold, extending down about the length of two vertebræ. Both of its margins fuse with the ventral wall, thereby forming a channel in the midline of the fold which unites with the central canal. In this specimen the spinal ganglia are 31 in number.

EMBRYO No. 875, 27 MM. CROWN-RUMP LENGTH.

In embryo No. 875 there are 34 vertebrae. The last is small and contains the winding part of the chorda dorsalis. The spinal cord narrows between the thirty-first and thirty-second vertebrae, as shown in figure 20. Its caudal end expands slightly and the extreme tip enters into the tail-bud. On the ventral wall of the central canal there are a few small folds. Near the caudal end of the vertebral column is a long, solid strand of cells, similar in structure to the cells of the spinal cord, which may have become separated from the latter at an earlier stage. Dorsal to the thirty-third and thirty-fourth vertebrae is a small papilliform tail, which is non-vertebrated and contains the caudal end of the vessels and a group of cells representing a remnant of the caudal end of the spinal cord. There are 31 spinal ganglia with nerve-fibers. The coccygeal tubercle and post-anal swelling are distinctly evident.

EMBRYO No. 75, 30 MM. CROWN-RUMP LENGTH.

At the caudal end of embryo No. 75 there is a small papilliform tail containing a group of cells which merge into the wall of the spinal canal, as shown in figure 43. The spinal cord narrows suddenly at the mid-level of the thirty-second vertebra, and its atrophic portion is further constricted at a level between the thirty-third and thirty-fourth vertebrae, as indicated in figure 43 (*constrict*). The part below this constriction is the primordium of the coccygeal medullary vestige and the upper part is destined in a later stage to undergo retrogression, leaving a small cell-sac as a second coccygeal medullary vestige. There are two large folds on the ventral wall of the spinal cord at a level with the thirty-first vertebra. In the median plane they are triangular in shape and consist of ependymal and mesenchymal cells that have been inverted, together with the wall. A large diverticulum lies between these two folds. The space below the folds probably represents the primordium of the ventriculus terminalis. Only the branches of the anterior spinal artery enter into these folds. There are 34 cartilaginous vertebrae, and at thirty-first and thirty-second vertebrae the column presents a typical curve. The chorda dorsalis shows a spindle-shaped swelling between the vertebrae, and is much convoluted at the caudal end, as seen in figure 43. There are 31 spinal ganglia; the nerves of the last pair are quite slender.

EMBRYO No. 145, 33 MM. CROWN-RUMP LENGTH.

Embryo No. 145 has 35 vertebrae, as diagrammatically shown in figure 22. The last one is situated on the dorsal side of the axis of the column, while the thirty-third and thirty-fourth lean towards the ventral side. There are 31 spinal ganglia, the thirty-second pair of nerves having no ganglia. In the caudal region there is a peculiarly shaped remnant of the neural tube, possibly an anomaly of development, which is connected with the main cord by a cell-strand. This cell-strand is directly continuous with the ependymal layer of the primordium of the ventriculus terminalis, and may possibly be regarded as the filum terminale. It emerges from the membranous sheath of the spinal cord, the more cranial portion branching irregularly, while the caudal portion bends dorsally to enter the minute tail-bud. The ends of the middle sacral artery and vein enter into the root of the tail. In this embryo no coccygeal tubercle can be seen.

EMBRYO No. 211, 33 MM. CROWN-RUMP LENGTH.

Embryo No. 211 has 34 vertebrae and 31 spinal ganglia. The vertebral column curves ventrally at the thirty-first and thirty-second vertebrae. The caudal end of the chorda dorsalis is undergoing regression and appears to be branching. The caudal end of the spinal cord may be divided into three portions: (1) the primordium of the conus medullaris, which includes the primordium of the ventriculus terminalis; (2) the filum terminale; (3) the coccygeal medullary vestige. The first extends about the length of the thirtieth

and thirty-first vertebræ, tapering gradually towards its caudal end. The ventriculus terminalis, which is included in the conus medullaris, expands in the medial part dorso-ventrally and transversely. The upper part of this cavity, which marks the entrance of the central canal, narrows slightly; the caudal end narrows sharply and forms a canal which terminates blindly at the end of the conus medullaris. The wall of the ventriculus terminalis consists of gray and white substance and the cavity is lined with a layer of ependymal cells. The dorsal wall is thicker than the ventral wall. The filum terminale extends from the caudal end of the conus medullaris, without definite boundaries, to a level between the thirty-second and thirty-third vertebræ. Its caudal end is represented by a slender bundle of nerve-fibers, and in its cranial portion there is a strand of ependymal cells. The large coccygeal medullary vestige is situated dorsal to the thirty-third and thirty-fourth vertebræ and its wall is thrown into a number of folds. At the caudal end it has two processes, one extending ventrally, the other dorsally. The latter enters into a rounded eminence at the caudal end of the embryo which represents a tail-bud, termed by Unger and Brugsch *caudal tubercle*. The post-anal swelling is well developed, while the coccygeal tubercle is scarcely to be made out.

EMBRYO No. 199, 35 MM. CROWN-RUMP LENGTH.

In number and development of its vertebræ embryo No. 199 is about the same as No. 972, description of which follows. The coccygeal vestige, however, shows greater expansion.

EMBRYO No. 449, 36 MM. CROWN-RUMP LENGTH.

Embryo No. 449 contains only 32 vertebræ, the last one being the smallest, as indicated in figure 24. The vertebral column shows a slight ventral curve at the point between the thirtieth and thirty-first vertebræ. The chorda dorsalis exhibits no convolutions at its caudal end. The spinal cord narrows at a level between the twenty-ninth and thirtieth vertebræ and the ventral wall of the atrophic portion presents a few folds. The remnant of the medullary tube was not found in the caudal region of this specimen. There are 31 spinal ganglia with nerves.

EMBRYO No. 972, 37 MM. CROWN-RUMP LENGTH.

Embryo No. 972 has 34 vertebræ, the last two having fused together at the center. The vertebral column curves ventrally at the thirtieth and thirty-first vertebræ, the curve being so sharp that the thirty-second, thirty-third, and thirty-fourth vertebræ are situated in a row nearly horizontal to the trunk, as can be seen in figure 44. Cranial to thirty-first the chorda dorsalis expands between the vertebræ. The caudal end is winding and broken.

At the caudal end of the spinal cord one can recognize the primordia of the conus medullaris, filum terminale, and coccygeal medullary vestige, as shown in figure 44. The primordium of the ventriculus terminalis, which is included in the conus medullaris, appears as a continuation of the central canal of the spinal cord without any line of demarcation, and is situated at a level with the twenty-ninth vertebra. Its ventral wall is thinner than the dorsal wall and shows a few small folds (fig. 44, x). The primordium of the filum terminale extends from the caudal end of the conus medullaris, viz, at the level of the under part of the thirtieth vertebra, to the middle of the thirty-second vertebra. It contains an incomplete canal which is lined by a remnant strand of ependymal cells which are directly continuous with the ependyma of the ventriculus terminalis. At its caudal end is an ependymal strand which is directly continuous with the coccygeal medullary vestige. In addition to this ependymal substance, there is a small bundle of nerve-fibers along the ventral border of the filum terminale which extends into the white substance of the cord above. The primordium of the coccygeal medullary vestige is situated dorsal to the last two vertebra and contains a slender cavity. There are 30 spinal ganglia supplied with complete nerves. The thirty-first ganglion has almost completely disappeared on each side, leaving the nerves exposed.

EMBRYO No. 362, 39 MM. CROWN-RUMP LENGTH.

Embryo No. 362 has 34 vertebræ, the last one being situated on the dorsal side of the vertebral axis, as is diagrammatically shown in figure 26. At the thirtieth and thirty-first vertebræ the column is bent ventrally. The caudal end of the chorda dorsalis winds and branches in the last three vertebræ. There are 30 spinal ganglia with nerves, but the thirty-first pair of nerves has no ganglia, their degeneration probably having occurred before that of the nerves. The caudal portion of the spinal cord is divided into the conus medullaris, filum terminale, and coccygeal medullary vestige. The ventriculus terminalis, which is included in the conus medullaris owing to the folding of its walls, is subdivided into two parts—an upper part, triangular in shape, and a lower, which is oblong and communicates with the upper by a narrow channel. The filum terminale reaches from the caudal end of the conus medullaris to the ventral side of the coccygeal vestige, being enveloped by the membrane of the spinal cord, the dura mater. This embryo presents a small tail-bud at its caudal end, containing a group of cells which connects with the coccygeal medullary vestige.

EMBRYO No. 95, 50 MM. CROWN-RUMP LENGTH.

Although embryo No. 95 is recorded in the catalogue of the Carnegie Collection as 46 mm. crown-rump length, its state of development more nearly corresponds with a 50 mm. embryo, and on this account I have used the latter measurement in the heading. This specimen has 35 vertebræ. The last one is very small and partly fused with the one above it. The column presents a ventral bend at the thirty-first vertebra, giving the typical coccygeal curve. The chorda dorsalis is disappearing in certain areas in the vertebral bodies as far down as the thirtieth vertebra, but in each intervertebral space a fragment remains. Caudal to the thirtieth vertebra the condition of the chorda remains the same as in the younger specimens, and in the thirty-second it gives off a short dorsal branch. The caudal end is more simple in form than in the younger stages, but I am inclined to believe that at an earlier stage it too was winding, as one can see in the thirty-fifth vertebra a few detached globules which probably at an earlier stage were continuous with the chorda and with it formed a terminal loop.

At the caudal end of the spinal cord are two groups of cells connected by a cell-strand. The more caudal one is situated dorsal to the thirty-fourth and thirty-fifth vertebræ; it is somewhat larger than the other, is oblong in form and incloses an oval cavity—a fragment of the central canal of the spinal cord. The other group of cells is situated dorsal to the thirty-second and thirty-third vertebræ and incloses a long, narrow cavity. The ventriculus terminalis extends the length of two vertebræ—the twenty-ninth and thirtieth. At this stage it has acquired its adult form. In none of the earlier specimens have I noted it so perfectly developed, although embryos No. 449, 36 mm., and No. 199, 35 mm., show a cavity at the caudal end of the central canal as the primordium of the ventriculus. In this specimen the structure is cylindrical in shape, has six walls, and measures 0.87 mm. long, 0.23 mm. deep, and 0.52 mm. wide. The ventral wall is concave, the dorsal convex, the sides slightly concave. The upper wall or ceiling is irregular and at the front presents a long, narrow diverticulum directed cranio-ventral. Behind this diverticulum is a narrow channel which connects the ventriculus terminalis and the central canal of the spinal cord. The ventriculus terminalis is embedded in the nerve-fibers of the cord. The filum terminale extends from the caudal end of the conus medullaris, at the level of the thirty-first vertebra, to a point between the thirty-third and thirty-fourth vertebræ, close to the column. It is covered by a membrane of the spinal cord and passes through the ventral side of the cell groups at the caudal end of the medullary tube. The pia mater covers closely the whole surface of the spinal cord; it contains blood capillaries, and is visible at the conus medullaris. The dura mater, which envelops loosely the pia mater, adheres

to the wall of the vertebral canal as far as the midlevel of the thirty-first vertebra, at which point it leaves the wall and unites with the caudal end of the conus medullaris. This portion constitutes the primordium of the bursa duræ matris. After the dura mater reaches the conus medullaris it envelops the pia mater quite closely, both following a caudal course and forming a sheath for the filum terminale. The point at which these membranes terminate can not be definitely decided. It is probable that the pia mater extends nearly to the end of the filum terminale between the thirty-third and thirty-fourth vertebrae. The fibers of the dura mater appear to enter into the caudal and dorsal portions of the last vertebra.

EMBRYO No. 184, 50 MM. CROWN-RUMP LENGTH.

Embryo No. 184 has 34 vertebrae, the last one being the smallest, as is indicated in figure 28. At the thirty-first vertebra the column presents a ventral curve, bringing the thirty-second, thirty-third, and thirty-fourth vertebrae in about a horizontal row and at right angles with the main column. The chorda dorsalis is disappearing in the 29 upper vertebral bodies, but at the thirtieth and below there is no change from the earlier stages, except that the chorda is relatively more slender. Its caudal end is bent caudo-dorsally before terminating; from this point the caudal ligament takes its origin. The middle sradral artery at this stage is a relatively delicate vessel, running from the ventral to the dorsal side of the vertebral column, and curving about the apex of the thirty-fourth vertebra. Its branches are plexiform, and in their meshes are groups of cells resembling neuroblast cells. The caudal end of the spinal cord contains a large cavity representing the ventriculus terminalis at a more advanced stage of development. The upper end of this cavity connects with the central canal of the spinal cord; its lower end terminates in two horns, the dorsal one of which is a blind pouch; the ventral horn is united with the caudal remnant of the spinal cord by a strand of ependymal cells and many transverse folds. The caudal remnant of the spinal cord consists of three separated portions. The first, which is attached to the caudal end of the ventriculus terminalis by an ependymal cell-strand, lies between the thirtieth and thirty-first vertebrae. This portion is embedded in nerve-fibers. As in younger specimens, it incloses a narrow cavity interrupted about midway. The second portion of the remnant is situated between the thirty-first and thirty-second vertebrae and leans to the dorsal side of the filum terminale. It also contains a small lumen. The third and largest portion is situated at the level of the thirty-third vertebra; its cavity is larger than the others and its caudal end enters into the caudal ligament.

The pia mater envelops the spinal cord and contains blood capillaries. It traverses the course of the filum terminale, completely inclosing it, and appears to reach the dorsal portion of the thirty-third vertebra, at which point the filum terminale ends. The dura mater also covers the spinal cord over the pia mater. At the caudal end of the conus medullaris, about the thirtieth vertebra, the dura mater adheres closely to the pia mater. At the dorsal side of the thirty-third vertebra the fibers of the dura mater merge with the fibers of the caudal ligament.

This embryo has 31 spinal ganglia on the right side and 30 on the left. The last ganglion on either side is very small, being in process of retrogression. The right thirtieth and thirty-first ganglia and the left thirtieth are not located between the vertebrae, but at the dorsal side of the upper vertebral bodies.

EMBRYO No. 448, 52 MM. CROWN-RUMP LENGTH.

A profile reconstruction of the caudal end of embryo No. 448 is shown in figure 45 and a more diagrammatic sketch is shown in figure 29. The embryo has 34 vertebrae, the last of which is only three-fourths the size of the thirty-third. The last three have begun to fuse, so that a section cut through the axis of the vertebral column shows one large vertebral body representing the three vertebrae, as shown in figure 45. The vertebral

column is bent ventrally between the thirtieth and thirty-first vertebrae, forming an obtuse angle and creating the typical coccygeal curve. Within the bodies of the vertebrae, from the first to the twenty-ninth, the chorda dorsalis is disappearing, but a remnant still remains in each intervertebral space. From the thirtieth to the thirty-fourth vertebrae it continues without convolutions, but the caudal end is branched and winding, partially disappearing at the dorso-caudal portion of the last vertebra close to the remnant of the spinal cord. The spinal cord tapers to a point as the conus medullaris and proceeds as the filum terminale from a level between the thirtieth and thirty-first vertebrae. Four portions of the neural tube can be distinguished at the caudal end of the spinal cord: (1) the sacral region of the spinal cord; (2) the conus medullaris and its contained ventriculus terminalis; (3) the filum terminale; (4) a remnant. The first consists of the ependymal zone, the mantle zone which contains the germinating nerve-cells, and the marginal zone, as is typical for the cord as a whole. The conus medullaris extends from the twenty-eighth to the thirtieth vertebrae, tapering gradually. In this region there is a large cavity, which in a median sagittal section shows four walls. Through the front of the upper wall the ventricle joins with the central canal of the spinal cord. The lower wall is narrow and from it extend two ependymal cell-strands. The longer of these goes straight downward to the first cell-group of the remnant of the medullary tube, through the axis of the conus medullaris. The shorter strand can be seen at the corner between the lower and ventral wall in figure 45. At the ventral wall is a diverticulum, the entrance to which appears as a narrow stalk consisting of a solid cord of ependymal cells and connecting with the ependymal cells of the cavity. This diverticulum is divided into two parts which are united by a cell-strand—a small upper sac and a larger lower sac. The ventral walls of both sacs are situated close to the surface of the conus medullaris, but do not open into it. The lower part of the conus medullaris consists chiefly of nerve fibers of the spinal cord, and here the central canal is entirely obliterated, leaving a long strand of ependymal cells. The conus medullaris extends to a point between the thirtieth and thirty-first vertebrae, and from there continues as the filum terminale, which extends to the last vertebra, skirting close along the dorsal side of the vertebral column. At the dorsal side of the filum terminale there are two remnants of the primitive neural tube. One of these is situated just dorsal to the apex of the conus medullaris. It contains a slender lumen, the remains of the central canal of the spinal cord. The other remnant (fig. 45, *ves. m. co.*) is situated dorso-caudal to the thirty-third and thirty-fourth vertebrae. It is oblong in shape and likewise contains a cavity, somewhat larger, which represents a remnant of the central canal. Its caudal end is sharp and fuses with the caudal ligament. The latter is not so distinct in this specimen as in the younger ones.

The caudal end of the sympathetic nerve-trunk lies between the middle sacral artery and vein, the three passing along the ventral side of the thirty-third and thirty-fourth vertebrae, where they curve around the apex of the last vertebra. The caudal ligament forms at the caudal end of the thirty-fourth vertebra and extends dorso-cranial to the coccygeal vestige. The caudal portions of the sympathetic trunks unite ventral to the thirtieth vertebra and become as one. After the union of these cords two additional ganglia can be seen—one at the thirty-first, the other at the thirty-third vertebra. From the latter the sympathetic nerve-trunk follows the midline of the vertebral column and curves around the last vertebra to the dorsal side, as shown in figure 45. The condition of the dorso-caudal portion of the nerve-trunk can not be clearly recognized.

The pia mater covers entirely the surface of the spinal cord and is rich in blood capillaries. It also envelops that portion of the filum terminale containing the cell-groups which connect with the ependymal cells of the ventriculus terminalis. The dura mater traverses the wall of the vertebral canal enveloping the spinal cord and its covering of pia mater. In the caudal region of the spinal cord there does not appear to be a distinct space

between the pia mater and dura mater and hence the arachnoid membrane is not visible at this point. A short distance from this, however, where the membranes envelop the conus medullaris, there is a marked space between the two membranes and here the arachnoid can be fairly well made out, forming a fibrous network of embryonic connective-tissue. At the level of the caudal third of the thirtieth vertebra where the filum terminale begins, the dura mater fuses with the pia mater and the two become separated from the wall of the vertebral canal and extend caudalward. The second group of cells, which lies caudo-dorsal to the thirty-third vertebra, does not seem to be covered by the pia mater or dura mater, these membranes having disappeared a short distance above.

EMBRYO No. 1656, 67 MM. CROWN-RUMP LENGTH.

There are 34 vertebrae in embryo No. 1656, the last being the smallest. At the thirty-first and thirty-second the vertebral column shows a ventral curve, the angle being sharper than in the younger specimens. The vertebrae are separated by embryonic tissue which is to develop at a later stage into intervertebral fibro-cartilage. This separation becomes progressively more marked above the thirtieth vertebra. Between the vertebrae which still lie close together is a small space where the chorda dorsalis coils as it emerges from the vertebral bodies in the median line. Several of these coils can be seen in figure 46, which is a profile reconstruction through the caudal end of the embryo. The blood-vessels enter the vertebral bodies from the ventral and dorsal side.

In the conus medullaris there are two medullary ventricles. The more cranially situated one is somewhat smaller than the other, measuring 0.55 by 0.25 by 0.33 mm. Its form, as seen in the sagittal plane, can be recognized in figure 46 (*vent. t. cran.*). The lower cavity is oblong in shape, measures 1.1 by 0.3 by 0.36 mm., and presents a canal-like appendage 1.7 mm. in length, as seen in figure 46 (*Append.*). This appendage tapers to a point and continues as a cell-strand. Toward the caudal end of the strand, in the path of the filum terminale, are two small groups of cells which represent the remnants of the ependymal cells of the medullary tube (fig. 46, *Re. epend.*).

The phenomenon of dedifferentiation at the caudal end of the spinal cord is well shown in this specimen. The appendage of the lower cavity was a complete ventriculus terminalis at the first stage; the main body of the cavity was a complete one at the second stage, and the upper cavity is the ventriculus terminalis at the present stage, thus showing a progressive upward trend. The gray substance which primarily existed around the ventriculus terminalis has now disappeared as the result of degeneration, and the caudal end of the central canal has gradually enlarged. The caudal end of the lower cavity, however, is becoming gradually narrow because the caudal portion of the conus medullaris, which contains the ventriculus terminalis, has also gradually become atrophied and lost its cell-like substances. The septum between the two cavities is a remnant of the gray substance of the spinal cord, in which the degeneration is not yet complete.

The filum terminale follows a downward course from the end of the conus medullaris and nerve-fibers can be recognized as far down as the caudal portion of the thirty-second vertebra. In the caudal region are found two cell-groups representing remnants of the neural tube; one, which lies between the thirty-second and thirty-third vertebrae, contains no lumen, and the epithelial cells are undergoing degeneration. The other is situated dorsally between the thirty-third and thirty-fourth vertebrae and incloses a small lumen.

The membranes of the spinal cord are more easily made out in this specimen than in the younger ones. The dura mater is separated from the periosteum of the vertebral bodies, especially at the ventral wall of the vertebral canal, by a dense plexus of blood-vessels, connective-tissue, and small spaces. This separation occurs at a level between the twenty-seventh and twenty-eighth vertebrae, and the dura mater becomes adherent to the conus medullaris between the twenty-eighth and twenty-ninth vertebrae, following an

oblique course from the periphery to the center of the vertebral canal. There is thus laid out the early form of the dural sac. Outside of this sac the fibers are separated into tufts which run parallel and caudalward. In the space between the dural sac and the conus medullaris the arachnoid membrane can be seen developing. The pia mater envelops closely the spinal cord and supports the blood-vessels; between the twenty-fifth and twenty-eighth vertebrae it is separated from the dura mater and the arachnoid by a still wider space.

EMBRYOS No. 662, 80 MM. CROWN-RUMP LENGTH; No. 928, 100 MM. CROWN-RUMP LENGTH;
No. 142, 125 MM. CROWN-RUMP LENGTH.

As the investigation with embryos Nos. 662, 928, and 142 was not very satisfactory, I shall not attempt to give its results in detail at this time. I have, however, made a special study of the coccygeal medullary vestige because of its importance in comparison with the same structure in younger specimens. In the 80 and 100 mm. embryos the coccygeal vestige is very small and its contained cavity narrower than in the younger specimens. In the 125 mm. embryo (negro) the structure is well developed and shows one long offshoot stretching under the epidermis at the sacral region. It is quite different in form and condition from the case reported by Tourneux (*Précis d'embryologie humaine*), and therefore does not present the loop formed by a more deeply situated limb (*segment coccygien direct*) and a more superficial limb (*segment coccygien refléché*). In this embryo the coccygeal vestige contains a slender cavity.

DEVELOPMENT AND REDUCTION OF THE TAIL.

In considering the process of reduction of the tail I should like, in the first place, to refer to the important study of this condition in mammals made by Braun (1882), whose conclusions in general are as follows:

(1) The tail of the mammalian embryo consists of two portions—a vertebrated part and a non-vertebrated part, the latter situated caudal to the former.

(2) The non-vertebrated part appears usually in the form of a thread at the end of the vertebrated tail, and consequently may be designated the caudal filament (*Schwanz-faden*). Being usually thinner than the tail itself, it is consequently sharply marked off from the latter.

(3) The vertebrated part of the tail can again be subdivided into two parts according to whether it projects from the body or not. The projecting part is designated as *tail*, although it has long been well known that this is directly continuous with the sacral vertebrae. The relative size of the internal and external tail varies, and hence we meet with long-tailed, short-tailed, and tailless mammals.

(4) The caudal filament is a transitory structure, although for a time it contains the end of the spinal cord, the chorda dorsalis, and the caudal gut. These structures undergo resorption and the last tissue to persist is the epidermis, the caudal thread for a time persisting of only epidermis cells.

(5) The caudal gut originally extends into the tail; before being resorbed it separates into fragments which disappear, the last to persist being the part constricted off at the tip of the tail.

(6) The chorda dorsalis always projects beyond the caudal vertebrae, where it separates into forked processes or curls in irregular loops. This part disappears completely.

(7) The spinal cord originally extends to the tip of the tail. The latter, however, soon exceeds it in length, when it terminates at the base of the caudal filament. It was possible to show in sheep embryos that the ascensus medullæ is due not alone to the overgrowth of the vertebrae, but that also there is degeneration and absorption of the caudal end of the spinal cord, to which in part the formation of the filum terminale owes its origin.

It would appear, therefore, that in his subdivision of the mammalian embryonic tail Braun included in the caudal filament that portion which lay between it and the vertebræ. My own position on the subject is briefly this: Is the caudal filament, through all the stages of mammalian embryonic life, one and the same thing as the non-vertebrated tail? That an intermediate portion exists between the two was apparently not recognized by Braun, but in man it constitutes a most important factor in the reduction of the tail vertebræ. After detailed investigation with the material at hand, numbering about 40 embryos ranging from 4 to 50 mm. in length, I was able to divide the caudal structure as follows: (1) the vertebrated portion; (2) the mesodermic end portion. In somewhat older embryos the first is subdivided into a proximal portion with persisting vertebræ, and a portion from which the primitive vertebræ have disappeared (lost-vertebræ portion). This point can better be understood by referring to the embryos themselves.

In embryo No. 221, 7.5 mm. long, the tail contains 38 somites and a long mesodermic remnant. The somites, which later develop into precartilaginous vertebræ, are well defined by the presence of small blood capillaries between them. On the dorsal surface of the tail the boundaries of the somites can be recognized distinctly as transverse shallow grooves. In the last somite, which is in contact with the mesodermic remnant, the boundary is not nearly so clear as in the others and would probably have disappeared altogether in the retrogressive process had the embryo lived. In this specimen the tail is entirely a vertebrated tail, as each somite is capable of development into a vertebra. The long mesodermic remnant at the caudal end, although separated by segmentation from the mesodermal sheet, evidently would not have developed into precartilaginous tissue. This part I have differentiated from the vertebrated tail as a mesodermic remnant, using the term employed by Keibel. I was able to distinguish in this embryo, therefore, two divisions of the tail—a long somitic portion, the vertebrated tail, and a short mesodermic portion, the caudal end of which may be compared with the caudal filament but not with the lost-vertebræ tail. In this stage the non-vertebrated portion has not as yet developed—that is, the portion in which the somites or precartilaginous vertebræ have disappeared. The last somite, however, shows signs of disappearing, and after a time, therefore, the lost-vertebræ portion will appear in place of the last somite. In other words, the reduction phenomenon has begun in the last somite; this progresses in the tail from one somite to the other, each losing its distinct boundaries, the blood capillaries fusing and disappearing.

In somewhat older specimens (8 mm., fig. 33), the last somite is larger than the preceding one and evidently represents the fusion of two pieces—the thirty-eighth somite and the mesodermic remnant. At the 11 mm. stage the lost-vertebræ portion of the tail becomes well developed (fig. 34). In the 12 and 15 mm. embryos the boundaries of the thirty-sixth, thirty-seventh, and thirty-eighth somites have become indistinct. In the 15.5 mm. specimen these three somites are converted into a cord which extends to the end of the tail (fig. 36, *str. cell*). This cord consists of embryonic cells which at an earlier stage of development existed in the somites as sclerotomes. In the median portion of the cell-strand are three

or four segments, and the last vertebra also shows two or three divisions. At this stage three types of vertebrae can be recognized at the caudal end of the vertebral column: (1) the vertebrae which have developed from the sclerotomes into precartilaginous or primitive vertebrae; (2) the incomplete primitive vertebrae, or the parts of the thirty-sixth and thirty-seventh sclerotomes which form the last vertebrae; (3) the cell-strand formed by the fusion of the last two somites and perhaps the thirty-sixth as well. I have not been able to determine whether or not the mesodermic remnant has merged into this strand. This mesodermic cell-strand—the primordium of the caudal ligament—is diagrammatically shown in figures 36 and 37 (*str. cell*). In the 16, 17, and 18 mm. embryos the last vertebra is larger than the more proximally situated ones and consists of two or three pieces united in the median plane; 35 vertebrae, developed into precartilage or cartilage tissue, were found in the 15.5, 17, and 18 mm. embryos, and 36 in the 16 mm. embryos. In those 17 mm. and larger the last two or three primitive vertebrae were usually found to be fusing at the center of the column, while in the lateral parts they show the divisions quite distinctly.

His did not find any extra vertebrae in the tail, but many other authors have recognized from 2 to 4. Perhaps the material upon which His based his studies did not include specimens in the same stages of development as my 15.5, 17, and 18 mm. embryos, in which the last vertebra consisted of two or three pieces. The 21 mm. embryo also represents the typical condition at this stage, the tail showing extra vertebrae. In this embryo can be clearly demonstrated a short tail consisting of two portions, such as has been described by His, the extreme non-vertebrated or, more correctly speaking, the lost-vertebrae portion, and the vertebrated portion. I am sure that embryos of this age never present a caudal filament homologous with that of other mammals, and I can not therefore agree with His, Braun, Keibel, Unger, and others, who describe the non-vertebrated portion of the tail in the human embryo as a caudal filament, since this portion at an earlier period contained somites capable of development into primitive vertebrae. Ecker, who studied human and mammalian embryos, asserted that the human embryo never has the caudal filament such as is the rule for mammalia. I could not recognize clearly a caudal filament in any of my specimens. In one of the three embryos (6.5, 7.5, and 8 mm.) which showed a portion of the caudal gut in the end of the tail, the caudal end was demarcated by a bend, and this might have been mistaken for a caudal filament. I am of the opinion that in the 8 and 10 mm. embryos the portion of the tail beyond the pointed end of the vertebral column, as shown in figure 34, can be compared with the caudal filament in mammals, but is not the true caudal filament described by Braun. It consists of the caudal end of the mesodermic remnant and contains the end of the neural canal and, in the 8 mm. embryo (fig. 33), a part of the caudal gut. I believe, also, that the non-vertebrated portion of the mammalian tail, which is not included in the caudal filament, is homologous with the non-vertebrated tail of the human embryo.

Having compared the non-vertebrated portion of the mammalian tail with that of human embryos, I have concluded that in the former the reduction process

occurs at an early stage, just as it does in the human embryo. This theory is based upon two facts: First, the last caudal vertebra is larger than the next proximally situated one, as in the cow embryo described by Braun, its increased size being due to the fusion of the last three pieces. This phenomenon represents the reduction process in the lower segments at a certain stage. Second, the number of vertebrae in the tail of sheep embryos, as asserted by Braun, is variable, and this variation must be due to a stronger or weaker effect of reduction.

In the 19 and 23 mm. specimens there is a very short tail with a caudal tubercle. In the 19 mm. embryo the vertebrae of the tail have fused together into one large vertebra—the thirty-third—in which can be recognized two or three pieces. The caudal end of the chorda dorsalis within this vertebra shows several coils, indicating a fusion of the last few vertebrae. The lost-vertebrae portion of the tail is represented in this specimen by the caudal extremity which contains the ends of the neural canal, the middle sacral artery and vein, and the chorda dorsalis. The latter adheres to the ventral wall of the neural canal and it appears as if the neural tube is retracted cranialward. In the 23 mm. embryo the development of the caudal end is farther advanced. The furrow between the tail root and the primitive anus becomes gradually more shallow, and the vertebral portion of the tail is embedded in the embryonic tissue which will later develop into the coccygeal tubercle. This shortening of the tail is evidently brought about by three factors: (1) fusion of the last few caudal vertebrae; (2) rapid growth of the alimentary canal and its surrounding structures; (3) the flexion of the caudal portion of the vertebral column.

(1) The disappearance of the last few caudal vertebrae by fusion, leaving only the winding end of the chorda dorsalis, is a well-established proof of the compression and final disappearance of the caudal vertebrae and the chorda dorsalis which was within them. Unger and Brugsch took the view that in spite of the presence of an external tail one could still speak of the formation of a coccygeal tubercle, inasmuch as the segments of the caudal region, which in their most caudal portion are already reduced, have begun to show a moderate, ventrally directed curve in their axis, which is eventually to be the coccygeal eminence. They point out that two factors are of importance in the formation of the coccygeal eminence: (a) the fusion (reduction) of the most caudal segments; (b) the bending in the axis of the caudal vertebrae. In the 25 and 27 mm. embryos the lost-vertebrae portion of the tail becomes rounded off and is shown as the caudal tubercle. Its extremity appears as a bud-shaped appendage and contains the caudal ends of the spinal cord, with its central canal, and of the middle sacral artery and vein. This bud-like appendage is called by many authors the *caudal filament*, but this is incorrect for the reason, as stated above, that it represents only a part of the lost-vertebrae portion of the tail which was primarily the vertebral portion, and therefore could never be considered as the caudal filament described by Braun.

(2) The area between the vertebral column and the rectum, especially the root of the tail, increases rapidly in a caudo-ventral direction. The caudal region of the rectum also extends down, its growth being in proportion to that of the vertebral column (figs. 39, 40, and 43). It is the belief of many authors—Rosen-

berg, Ecker, Keibel, and others—that the tail and the coccygeal tubercle in human embryos become shorter and finally disappear by an increase in volume of the caudal soft tissues, muscular tissue, subcutaneous connective-tissue, etc., which surround the caudal part of the vertebral column.

At an earlier stage the swelling between the primitive anus and the root of the tail is called the post-anal swelling. Keibel asserts that in embryos 11 mm. and larger the root of the tail is separated from the ventral trunk by double plates of epithelial cells which lie between it and the anus. Therefore, by means of these plates, which consist of two sheets of epidermal cells connected ventrally to the post-anal epidermis and dorsally to the ventral side of the tail, the tail-root is distinctly marked off. Following Keibel's idea, Tourneux speaks of it as *dépression sous caudale de l'intégrument externe*. Unger and Brugsch describe the stages of disappearance of this post-anal swelling in embryos 25 and 45 mm. long. In my specimens it is quite clear. In the 13 and 14 mm. embryos these plates are visible, but in the 45 mm. specimen they have disappeared. After observing the specimens in the various stages, my conclusions on this point are as follows: At a certain stage (13 mm. and older) the digestive tube grows more rapidly than the vertebral canal, so that the depression gradually straightens out. At this time the caudal region of the digestive tract—viz, the cloacal region—develops faster than the caudal end of the vertebral column, which constitutes the caudal end of the internal tail. Moreover, the mesodermic tissue between the primitive anus and the root of the tail develops rapidly and gradually bulges downward. By the swelling of the caudo-ventral region of the tail-root the fold of epidermis, or so-called epithelial plate, is stretched by degrees and at last disappears. The growing of the coccygeal tubercle would also aid in this process. In his paper Keibel asserts that the mesodermic tissue between the primitive anus and the tail-root grows luxuriantly at certain stages and bulges downward. He terms this swelling *die postanalen wulst* (post-anal swelling). In this way the epithelial plates disappear. This epidermal plate between the anus and tail-root moves gradually caudalward. In the 12 mm. embryo it is situated at the level of the thirty-third vertebra, and in the 46 mm. specimen has moved down to the level of the thirty-fourth vertebra. The caudal end of the rectum—viz, the caudal end of the digestive tract, and perhaps that of the genito-urinary organs as well—has likewise moved caudalward.

(3) Originally the caudal portion of the vertebral column is nearly a straight line, but in embryos about 20 mm. long the axis of the column shows a distinct ventral flexion at about the level of the thirtieth or thirty-first vertebra. There is a second flexion which is dorsal at the caudal end of the vertebral column, between the vertebrated portion and the lost-vertebræ portion of the tail, which is seen in younger embryos. The caudal remainder of the lost-vertebræ tail has, therefore, moved to the dorsal side of the vertebral column, being joined to the last vertebra by bands of embryonic connective-tissue. These bands are the so-called caudal ligament. In these embryos the ridge or epidermal plate between the coccygeal tubercle and the rectum has become shallow almost to the point of disappearance, as shown in figures 40 and 42. In the 30, 33, and 39 mm. embryos the lost-vertebræ portion of the tail has almost entirely disappeared from the sur-

face of the skin. In the first embryo the tail remnant is surrounded by a minute furrow, while in the 33 mm. specimen it appears as a rounded eminence; and finally, at the 39 mm. stage, the remnant of the tail is represented by a small papilla. These remnants contain groups of cells from the primitive neural canal. The apex of the caudal conical eminence, the *caudal tubercle*, according to Unger and Brugseh, in which the cell-strand of the neural canal enters, is a part of the lost-vertebræ tail, or so-called non-vertebrated tail. The various stages in the reduction of the tail as shown on the skin surface do not present the same appearance in every embryo; but on section evidences of its reduction and disappearance are invariably found dorsal to the caudal end of the vertebral column—that is, dorsal to the coccygeal tubercle and in the median line of the embryo. I have seen no case in which the remnant of the tail is situated just at the caudal end nearly in line with the extended axis of the vertebral column—namely, at the top of the coccygeal tubercle. At this stage the caudal ligament is well developed and consists of bands of connective-tissue. The curve of the vertebral column is quite prominent at the thirtieth, thirty-first, and thirty-second vertebræ. This flexion of the caudal portion of the column begins at about the 25 mm. stage, although sometimes it does not appear until the 33 mm. stage. These embryos show a small tail at the caudo-dorsal end. In the 30 mm. embryo (fig. 43) and the 39 mm. embryo, where the tail is disappearing from the surface of the skin, this curving of the vertebral column becomes more marked than in the younger specimens.

Concerning the disappearance of the tail in the human embryo, I am of the opinion that, while the lost-vertebræ portion of the tail disappears from the skin surface, a few vertebræ of the tail fuse with the one above, usually the thirty-fourth, a part of which disappears by dedifferentiation; and that the caudal portion of the column, which consists of the thirty-first, thirty-second, thirty-third, and thirty-fourth vertebræ, is bent to the ventral side, sinking into the embryonic tissue between the rectum and the coccygeal tubercle. After the tail entirely disappears there appears outside of the ventral region of the tail root a blunt conical eminence known as the coccygeal tubercle, or *eminentia coccygealis*. This tubercle is a temporary swelling formed by bulging of the caudal end of the vertebral column and the addition of embryonic tissue contained in the lost-vertebræ tail at an earlier stage. The tubercle disappears at some time between the 33 and 52 mm. stage, while the caudal end of the vertebral column, the so-called internal tail (after Braun), sinks deeper into the soft tissues which surround and envelop it (figs. 44 and 45).

In describing the embryonic tail in mammals, Braun divides it into internal tail (*die innere Schwanz*) and external tail (*aussere Schwanz*). This theoretical arrangement may be the better one. In the human embryo, at least in my specimens, it can be clearly demonstrated. In an embryo of 21 mm. one can recognize the external tail, which may be divided into two portions—vertebrated and non-vertebrated. In the 27, 30, and 39 mm. specimens the thirty-first, thirty-second, thirty-third, and thirty-fourth vertebræ belong to those of the internal tail. I agree with other authors that the human embryo has a true tail at a certain stage of its development and that the second coccygeal vertebræ and those caudal to it in the adult are the true tail vertebræ in the philogenetic sense.

CHORDA DORSALIS.

In the 4 mm. embryo the chorda dorsalis lies close to the ventral side of the neural tube, but cranial to the twenty-first segment it is separated from the tube by the tissue of the primitive vertebræ. At this stage it forms a long, narrow tube, its caudal end consisting of only a small cell-strand which terminates in a cell-mass above the caudal extremities of the neural tube and caudal gut. In the 5 mm. embryo, which is shown in figure 31, the chorda cranial to the thirty-third somite is separated from the ventral side of the neural tube, while caudal to the thirty-third the two are contiguous. The chorda terminates in the mesodermic remnant, being covered by the ventral wall of the neural tube, and at its caudal end is united with that of the caudal gut by a cell-strand. In specimens 7.5 to 11 mm. the greater part of the chorda dorsalis cranial to the thirty-fourth or thirty-fifth somite is embedded in the primitive vertebral column and shows considerable winding. Caudal to the thirty-first somite the chorda is placed between the neural canal and the primitive vertebral column. In passing down through the column it shows a series of segmental undulating curves—that is, it alternately bends ventrally and dorsally. The dorsal bends occur at the foci of vertebral formation which eventually become the intervertebral spaces. In older embryos—12 to 14 mm.—this segmental undulation of the chorda gradually disappears. In the 12 mm. embryo (as shown in figure 35) the chorda is more completely embedded in the column, although here its terminal portion emerges to lie in the space between the spinal cord and the tissue of the column. As the embryo advances in age this bending of the chorda gradually decreases, until at about the 18 mm. stage it becomes straight in its main portion, while the caudal part, which was hitherto straight, now becomes curved, the first indication of the formation of undulations (compare figs. 35, 36, 37, and 39), which, however, are probably not segmental like those above described, but are a phenomenon of the process of reduction in the caudal primitive vertebræ.

When we compare the 7.5, 8, and 11 mm. embryos with those from 15 to 19 mm. it is easy to see that at first the few caudally situated primitive vertebræ—the scleromeres—fuse together, and the chorda which is within them becomes convoluted and recedes cranialward. The winding portion of the chorda is, therefore, situated in the last vertebra which has developed by the fusion of several vertebræ. This condition remains the same up to the 18 mm. or even more advanced stage, and finally, in the 23 mm. stage the chorda presents a spiral appearance, as shown in figure 40. Braun found this same process in sheep and other mammalian embryos, the end of the chorda projecting caudalward from the last vertebra. Ecker also noted this projection of the chorda in his mammalian material. These authors recognized the winding or branched end of the chorda in the caudal filament or in the extreme end of the tail and concluded that this was its primitive state. In human embryos, however, as mentioned above, at the earliest stage when the chorda reaches the extreme end of the tail, its caudal end is straight and shows no winding until the reduction of the tail begins. In embryos from 15 to 23 mm. the caudal end projects almost caudalward from the last vertebra which has been formed by the fusion of several vertebræ. His did not find such a condi-

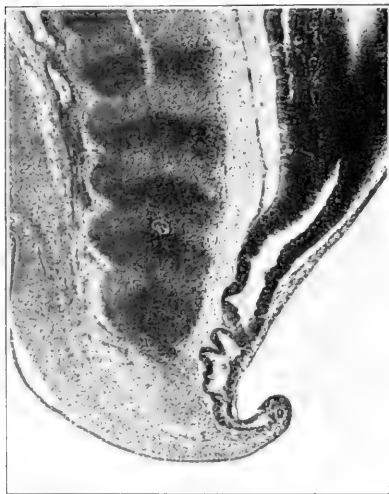
tion in human embryos, although it is usual. According to Braun, the occurrence of a free, naked end of the chorda is due to the disappearance of the last primitive vertebræ by which it had previously been surrounded. In the human embryo the caudal end of the chorda was never surrounded by primitive vertebræ, but was situated between the neural canal and the primitive vertebral column. In the 25 mm. (fig. 42) and the 30 mm. embryo (fig. 43) the coil-like appearance of the chorda is typical, and these stages, therefore, are the clearest of any throughout embryonic life. Later on, for example, in the 37 mm. embryo (fig. 44), the caudal end of the chorda is disappearing, leaving a few remnants which have become separated from the main chordal strand. This degenerative fragmentation and partial absorption of the terminal portion of the chorda results in a great variety of forms. Very seldom is the caudal end branched in the earlier stages. From the 39 mm. stage the chorda becomes gradually reduced and is finally converted into a more simple form, as shown in figures 45 and 46. While the short caudal portion shows the above-mentioned variations, the main strand changes but slightly. After it becomes straightened some portions of it which lie in the intervertebral fibro-cartilage show spindle-shaped swellings, as shown in figures 39 and 42 (18 to 25 mm.). At last, in the 50 and 52 mm. embryos, the parts embedded in the vertebral bodies disappear, leaving small remnants in the intervertebral spaces. Frequently these remnants show visible coils, as can be seen in figure 46. These remain often until a later stage. The disappearance of the chorda below the thirtieth vertebra occurs later than that of the main strand, and we can therefore still recognize it in the 67 mm. embryo as a continuous cord through the caudal vertebral bodies.

DEVELOPMENT OF THE CAUDAL END OF THE SPINAL CORD.

In the 4 mm. embryo the caudal end of the neural tube fuses with the solid mass of mesodermal cells which extends to the ventral side of the tail. The caudal ends of the chorda dorsalis and caudal gut also merge with this cell-mass. In the 5.5 to 7.5 mm. embryos the caudal end of the neural tube, with its central canal, extends to the apex of the tail and merges into the mesodermal cell-mass, entirely losing its boundaries. In the 8 mm. specimen a difference can be plainly recognized between the caudal portion of the spinal cord and the portion that lies cranial to the thirty-second somite. Caudal to this level the central canal is distinctly narrower. Thus it may be divided into two portions, an upper, wider canal and a caudal narrow or atrophic canal. The former constitutes the main part of the central canal of the spinal cord. On cross-section it is oval in shape and its walls show no folds. The caudal part is narrower in its dorso-ventral diameter than is the main canal, and therefore on section presents a more rounded form. Sometimes a large fold is found between the two parts and in the 11 and 12 mm. specimens can be seen the primordia of other folds on the walls of the atrophic canal, especially on the ventral side, as shown in figures 34 and 35. The distinction between the atrophic portion of the spinal cord and the main part is quite marked in the 15.5, 16, 17, and 19 mm. embryos. The caudal end of the wider canal expands transversely, and where it narrows into the atrophic canal constitutes the

initial form of the ventriculus terminalis. The portion of the spinal cord which incloses the ventriculus is the primitive conus medullaris.

The ventriculus terminalis was found by Argutinsky in a 45 mm. embryo; by Brugsch and Unger in a 25 mm. embryo, and what may be considered as its primordium is already apparent in my specimens of 11 and 12 mm. respectively, as shown in figures 34, 35, and 36. It can be seen from stage to stage retreating cranialward, while the atrophic canal gradually lengthens. In the 18, 23, and 25 mm. embryos the latter expands noticeably in the median or caudal region, as shown in figures 39, 40, and 42. This was also observed by Unger and Brugsch, who, however, did not regard it as the primordium of the ventriculus terminalis, but rather as a homologue of the sinus terminalis of the amphibians, which develops at the caudal end of the central canal of the spinal cord. In the majority of my specimens from 18 to 30 mm. the caudal end of the atrophic canal shows diverticula such as those described by Unger and Brugsch. In such embryos the spinal cord becomes



TEXT-FIGURE 1.

Sagittal section through caudal end of a human embryo 18 mm. long (Carnegie Collection, No. 432, slide 22, row 1, sec. 3), enlarged 50 diameters. The wall of the atrophic portion of the spinal cord is thinner and represents a much younger stage of development than that of the main part of spinal cord. From this time on it undergoes gradual regressive changes with obliteration of the central canal and is eventually replaced by a simple fibrous strand.

temporarily longer than the vertebral column. It seems probable, therefore, that in the wall of the atrophic portion of the cord the ependymal cells increase rapidly by proliferation, and perhaps also by the migration of other ependymal cells from the more caudal part of the tail, which is in process of regression. By reason of these two processes folds develop in this wall, such as are well shown in text-figure 1. In these embryos the caudal end of the chorda dorsalis seems to exert an attraction upon the caudal end of the medullary tube, thus drawing it into a more cranial position (fig. 39). In embryos of 30, 33, 35, and 37 mm. the atrophic canal is longer and narrower than in the slightly younger specimens, but the caudal end is still dilated. At several points the canal has become so narrowed that its central cavity is obliterated and gradually becomes converted into a cell-strand, as shown in figure 44.

At the stage where the embryo has entirely lost its external tail the spinal cord is about the same length as the vertebral column, as shown clearly in the 30 and 37 mm. specimens. From this time on the vertebral column increases relatively in length, although there is no cessation of growth of the spinal cord. In the 37 mm. embryo a bundle of nerve-fibers (*i. e.*, marginal zone) is visible on the ventral side of the atrophic cord, as shown in

figure 44 (*fil. t.*), and represents a primitive filum terminale. This structure extends caudalward from the apex of the primitive conus medullaris at a level between the twenty-ninth and thirtieth vertebræ. In the 33 mm. embryo the cranial portion of the atrophic canal and its ependymal lining disappear and the bundle of nerve-fibers remains in the sheath of the dura mater. The dilated portion of the atrophic canal remains as the coccygeal vestige. The process of dedifferentiation of the caudal end of the spinal cord, viz, the reduction of the cranial end of the atrophic canal, advances step by step, so that what appeared as a long, narrow tube in the 37 mm. embryo is divided into several parts in the 50 mm. specimen, each part containing a cavity, the more cranially situated part being joined to the ventriculus terminalis by a cell-strand which is destined also to disappear in the course of development (figs. 44, 45, and 46). The ventriculus terminalis, which had only begun in the 37 mm. specimen, has developed completely in one of 50 mm. Its formation is the result of the gradual constricting of the expanded area of the central canal which marks the division between its upper wider and lower atrophic portions, and a separate cavity is thus formed. Complete fusion of the margins, however, does not occur, a narrow channel being left which connects the two portions of the central canal. In their thesis Brugsch and Unger have described in detail this process of reduction of the central canal. In my specimens the phenomenon is first noted in the 39 mm. embryo. In the 39, 46, 50, and 52 mm. specimens the conus medullaris, ventriculus terminalis, and filum terminale are quite distinct, and the remnant of the neural tube caudal to the filum terminale persists as the coccygeal medullary vestige. The form of the ventriculus terminalis varies in the different specimens, while that of the conus medullaris is much the same in all. In the specimens above enumerated the ventriculus terminalis is situated at about the level of the twenty-ninth or thirtieth vertebra, the position of both it and the conus medullaris gradually becoming more cranial as the result of the fact that the growth of the vertebral column becomes progressively more rapid than that of the spinal cord. In these embryos, especially the 46 mm. specimen, the membranes of the spinal cord—the dura mater and pia mater—are visible. At a level with the upper border of the thirty-first vertebra, in the 46 mm. embryo, the dura mater may be seen to leave the wall of the vertebral canal for the caudal end of the conus medullaris, which marks the beginning of the filum terminale, thus forming a sheath for the latter.

As a rule, the coccygeal medullary vestige is on the dorsal side of the last two vertebræ. It is situated in the connective-tissue surrounding the vertebræ and does not adhere to the epidermis. Tourneux and Hermann discovered the caudal remnant of the spinal cord in a 37 mm. embryo and termed it the *vestiges médullaires coccygiens*. Tourneux advanced the theory that the slightly enlarged caudal tip of the neural tube is closely united in the deep layers of the skin. Toward the end of the third month the spinal column, developing more rapidly than the soft parts, draws along the part of the neural tube adherent to it, the extreme tip of which remains attached to the skin. As a result of this the terminal or coccygeal portion of the neural tube becomes bent in the form of a loop, the more deeply situated limb being termed *segment coccygien direct*, and the more superficial one

segment coccygien réfléchi. In my specimens I did not find such to be the case, nor was it noted by Unger and Brugsch. I believe, therefore, that the condition noted by Tourneux is of rare occurrence. In the 39 mm. embryo may be seen a small papilliform tail, at the root of which is a group of cells representing the remnant of the spinal cord. The caudal end of the coccygeal medullary vestige appears to adhere to the epidermis, but in reality does not, although Tourneux and Hermann found that it did adhere in their case.

Concerning the development of the coccygeal medullary vestige from the remnant of the neural tube, I am led to the following conclusions:

(1) The expanded caudal end of the neural tube in an embryo in which the tail has disappeared is the primordium of the coccygeal vestige (figs. 40, 42, and 43).

(2) In addition to the coccygeal vestige there frequently occurs a similarly formed epithelial sac situated in a more cranial position.

(3) The caudal end of the coccygeal vestige merges into the caudal ligament, as believed by Brugsch.

(4) In the younger specimens the fibers which persist as the filum terminale always lie ventral to the ependymal cells, which become the coccygeal vestige.

(5) The middle sacral artery and vein extend to and curve around the apex of the coccygeal vestige.

(6) Only in rare cases is the coccygeal vestige lacking. In my entire series of specimens, from 4 to 125 mm., in only one did I actually fail to find it (fig. 24).

(7) In specimens from 4 to 100 mm. the coccygeal vestige is not adherent to the epidermal layer of the skin.

(8) The coccygeal vestige continues to grow after the 100 mm. stage.

In the 67 mm. embryo, as can be seen in figure 46, the ventriculus terminalis occupies a more cranial position than in the younger specimens; the conus medullaris has become relatively more slender and the filum terminale longer, the latter disappearing caudal to the thirty-second vertebra, two remnants of the neural tube being left. The membranes of the spinal cord are here also considerably further developed than in the younger specimens. At this stage the arachnoid membrane lies between the dura mater and pia mater. At the upper border of the twenty-seventh vertebra the dura mater leaves the wall of the vertebral canal for the filum terminale, forming a sheath and reaching the filum terminale at a level between the twenty-eighth and twenty-ninth vertebrae. In younger specimens, for example in the 46 mm. embryo, this separation occurs at a level with the thirty-first vertebra. Therefore, the caudal end of the dural sac, as well as the spinal cord, recedes cranialward. This phenomenon is an evidence of the relatively more rapid growth of the vertebral column. What, then, is the cause of the lengthening of the filum terminale? In the 33 mm. embryo I could recognize distinctly, below the conus medullaris, a bundle of fibers representing a primitive filum terminale. In the 37, 39, and 50 mm. embryos this reaches almost to the caudal end of the neural tube or the coccygeal medullary vestige. In the 37 and 39 mm. specimens it extends farther caudalward than in any of the others (fig. 44). It is my opinion, therefore, that the filum terminale consists at an early

stage of nerve-fibers, especially those from the ventral portion of the spinal cord, although von Kölliker does not hold this view. After the development of the ventriculus terminalis the caudal portion of the conus medullaris is converted into the filum terminale by the ventriculus terminalis and conus medullaris moving cranialward. This is due to the fact that the gray substance which lies next to the ventriculus terminalis is undergoing degeneration and the caudal end of the central canal is gradually excavated, while the caudal end of the ventriculus terminalis narrows by degrees, losing its cellular substance. The relative lengthening of the filum terminale, therefore, is due to the growth of the nerve-fibers with their sheath of dura mater and pia mater, and in part also to the gradual addition of tissue from the caudal portion of the conus medullaris, which has become converted into the tissue of the filum terminale.

ABNORMALITIES OF THE CAUDAL END OF THE SPINAL CORD.

(a) Embryo No. 405, 26 mm.; (b) embryo No. 145, 33 mm.; (c) embryo No. 449, 36 mm.

In the first two specimens the caudal tip of the neural tube is spiral. It is probable that this part is covered with a layer of epidermis, although I could not discover it and therefore conclude that it was injured in the preparations. In the first specimen the caudal end of the spinal cord enters into the tail-bud. Two branches of the anterior spinal artery penetrate between the coils of the spinal cord. In the second specimen the coil of the caudal end of the spinal cord forms the summit of the papillary tail and terminates at the ventral side of its root. The third specimen has only 32 vertebræ and no remnant of the neural tube.

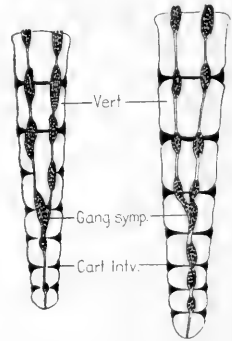
SPINAL GANGLIA.

It is very difficult to locate the first cervical ganglion at a very early stage of embryonic development, particularly if the specimen is poorly preserved. This structure is frequently found in close apposition to the Froriep ganglion on the trunk of the spinal accessory nerve. Sometimes it is poorly developed and resembles a Froriep ganglion, except for the fact that it lies always on the ventral side of the trunk of the accessory nerve, while the Froriep ganglion lies on the dorsal side. The first cervical is smaller than the others, and the second in turn is smaller than the third. In embryo No. 991 (17 mm.) both first cervical ganglia are lacking. In embryos from 5 to 10 mm. there are in most cases 32 pairs of ganglia; from 12 to 14 mm. there may be 33 pairs. When the number is 33 the last caudal ganglion is usually very small and has no nerve. In embryos from 15 to 33 mm. the number is usually 31. I have frequently found the thirty-second spinal nerve without a ganglion, the latter having degenerated. In embryos from 35 to 67 mm. long, and older, there are usually only 30 ganglia; occasionally there may be 31, but the last is usually undergoing degeneration.

SYMPATHETIC GANGLIA.

In embryos 33 mm. long, and older, the caudal ends of the paired sympathetic ganglionated nerve-trunks join together at the upper plane of the ventral side of the thirtieth vertebra, as shown in text-figure 2. At the point of union there is

usually found a ganglion; another occurs approximately at a point between the thirty-first and thirty-second vertebrae. From the latter ganglion the single nerve-trunk follows the course of the middle sacral artery and vein, running between them, and emerges dorsally from the caudal end of the vertebral column, where the coccygeal medullary vestige and caudal ligament curve about the apex of the column. This nerve consists of a large bundle of non-medullated nerve-fibers, but the structure of its caudal end can not at this stage be made out distinctly. At a level between the thirty-third and thirty-fourth vertebrae, or perhaps a little above, there is a small group of cells representing a sympathetic ganglion. At this point are frequently found numerous plexiform branches of blood-vessels enmeshing this group of cells. This richly vascularized cell-group may be the primordium of the glandula sacralis.



TEXT-FIGURE 2.

Ventral view of caudal portion of vertebral column, showing characteristic arrangement of sympathetic ganglion-strands in human embryos between 46 and 67 mm. long. *Cart. intv.*, intervertebral fibrocartilage; *Gang. symp.*, ganglionic node; *Vert.*, body of vertebra.

SUMMARY.

(1) The human embryo possesses a true tail composed of primitive vertebrae and the caudal ends of the spinal cord, chorda dorsalis, and middle sacral artery and vein.

(2) The longest and most completely developed tail among the specimens examined by me was found in a 7.5 mm. embryo. This was 1.2 mm. in length.

(3) The human embryo does not possess a caudal filament homologous with that of other mammals.

(4) The reduction of the tail, especially of the primitive vertebrae, begins when the embryo has reached a length of about 8 or 9 mm.

(5) Prior to this the tail consists of two parts: a proximal longer part (the vertebrated tail), which has well-formed somites, and a caudal shorter part which contains only a mesodermic remnant.

(6) In embryos from 25 to 27 mm. the tail is reduced to a small papilla, in which are contained the caudal ends of the spinal cord and the middle sacral artery and vein, and into which the end of the caudal ligament enters. As a rule this tail-bud is not situated directly at the caudal extremity of the vertebral column, but slightly dorsal to it. The vertebrated portion of the external tail has retracted into the soft tissues and has thus become an internal tail, whereas the lost-vertebrae tail projects temporarily and finally it also disappears.

(7) At the time the division of the external tail takes place two eminences appear at the caudal region; one ventral (coccygeal tubercle or Steisshöcker), the other dorsal (caudal tubercle or Kaudalhöcker). The first is due to the pushing up of the caudal end of the internal-tail vertebrae, formerly situated in the root of the external tail and constituting the vertebral portion of it in younger embryos. The second is usually shaped like a small papilla and by some authors is called the tail-bud or caudal filament, although the latter, as stated above, is entirely a

misnomer. Sometimes it appears as a rounded eminence and was therefore termed caudal tubercle by Unger and Brugsch.

(8) The tail-like appendage that occasionally persists in adults may possibly be explained as a persistent caudal tubercle that did not undergo the normal reduction. It must be granted, however, that in none of the cases reported has osseous or cartilaginous tissue been found.

(9) When the embryo reaches 30 to 35 mm. the tail has usually entirely disappeared, although the time of disappearance is quite variable. Thus, the tail was found to have disappeared in the 24 mm. embryo, while in one 39 mm. long it still persisted.

(10) In embryos above 40 mm. in length that have lost the external tail I have designated as the internal tail the portion caudal to the thirtieth vertebra, for three reasons: (a) the curve of the vertebral column occurs at the thirty-first and thirty-second vertebrae; (b) below the twenty-ninth vertebra the spinal ganglia disappear at about the same time as the external tail; (c) the sympathetic ganglion strands unite between the thirtieth and thirty-first vertebrae.

(11) The disappearance of the canal of the caudal gut had already begun in a 5.5 mm. embryo and in a 6.5 mm. specimen the caudal gut had become converted into a long cell-strand, except for a short caudal portion. In embryos 7.5, 8, and 9 mm. the remnant of the caudal gut, inclosing a small cavity, was still found in the end of the tail. In those 10 mm. and older the caudal gut had entirely disappeared.

(12) In the very youngest specimens the medullary tube reaches to the extreme tip of the tail.

(13) In those 11, 12, and 15.5 mm. long the medullary tube can be divided into two parts at the level of the thirty-second vertebra: a cranial part, having a wide central canal, and an atrophic caudal part with a narrow canal. This distinction becomes quite marked in the 15.5 mm. specimen. The canal at the junction of these two parts is slightly enlarged transversely and constitutes the primordium of the ventriculus terminalis.

(14) The atrophic portion of the spinal cord gradually becomes more slender, although its caudal end remains unchanged for some time or in some instances shows temporary enlargement. It later subdivides, the cranial end forming the cell-strand of the filum terminale and the caudal end developing into the coccygeal medullary vestige.

(15) The caudal end of the wider part of the spinal cord develops into the conus medullaris and its lumen constitutes the ventriculus terminalis.

(16) In the 46 mm. embryo the ventriculus terminalis is perfectly developed.

(17) The conus medullaris and the ventriculus terminalis recede cranialward as the result of two processes: (a) the growth of the vertebral column, which is more rapid than that of the spinal cord; (b) the degeneration of the gray substance which forms the upper wall of the ventriculus, thus causing the cavity to enlarge and gradually move upward while its caudal end narrows.

(18) The extent of the coiling of the chorda dorsalis in its various stages indicates the extent of fusion of the last primitive vertebrae.

(19) In embryos from 12 to 14 mm. the spinal ganglia are 33 in number, but at about this period reduction begins in the more caudally situated ones. Thus in an embryo of 67 mm. there are but 29 ganglia.

In conclusion, I take this opportunity to acknowledge my indebtedness to the late Professor Franklin P. Mall for the privilege of using the valuable material in the collection of human embryos belonging to the Carnegie Institution of Washington. I also wish to thank Dr. George L. Streeter for his kind assistance in the preparation of this paper.

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DESCRIPTION OF PLATES.

(The description of Plate 1 will be found on the plate itself.)

The figures on Plates 2 to 4 represent profile reconstructions of the caudal region in a series of human embryos selected from the Carnegie Collection. The different structures are indicated by the following abbreviations:

<p>A. s. m. Arteria sacralis media. Append. Caudal extension of the terminal ventricle. Caud. non-v. Part of tail containing no vertebrae. Ch. Chorda dorsalis. Cl. Cloaca. Constrict. Constriction marking off remnant of spinal cord which is to form the coccygeal medullary vestige. Con. med. Conus medullaris. Dura. Dura mater. Fil. t. Filum terminale. Gang. symp. Sympathetic ganglionated cord. Int. cau. Intestinum caudale. Lig. cau. Ligamentum caudale. Med. sp. Medulla spinalis. Med. sp. atr. Atrophic portion of the spinal cord. Mem. cl. Membrana cloacalis.</p>	<p>Pia. Pia mater. Pl. vasc. Plexus vasculosus. Re. epend. Remnant mass of ependymal cells. Reet. Rectum. Re. int. cau. Remnant of caudal gut. Rud. cau. Rudiment of tail. Str. cell. Stria cellularis. Tub. co. Coccygeal tubercle. V. s. m. Vena sacralis media. Vent. t. Ventriculus terminalis. Vent. t. cran. Cranial portion of terminal ventricle. Vent. t. cau. Caudal portion of terminal ventricle. x. Characteristic folds in ventral wall of spinal cord. xx. Furrow on surface corresponding to level at which the caudal gut begins to disappear.</p>
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PLATE 2.

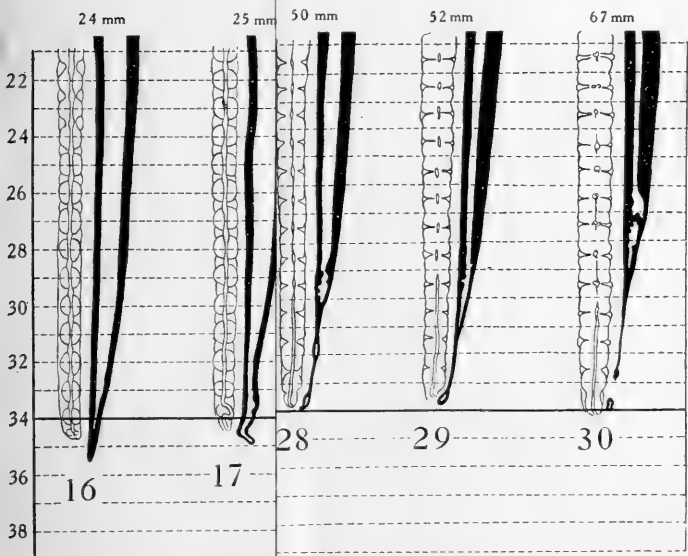
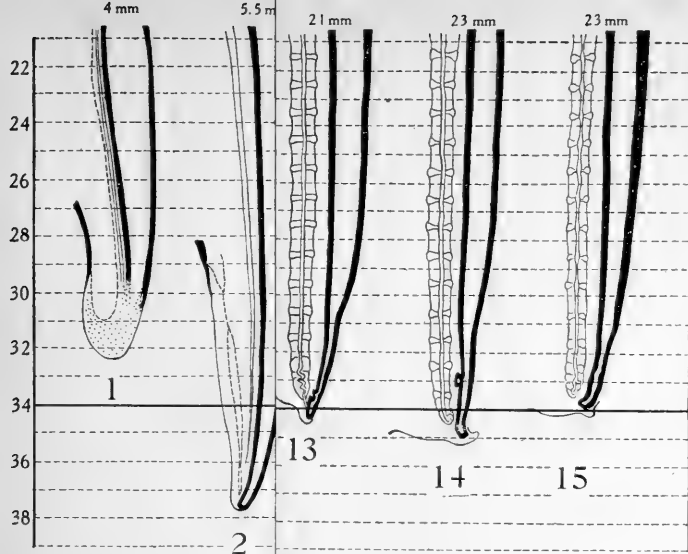
- FIG. 31. Embryo No. 810, 5.5 mm., enlarged 31.5 diameters. The caudal gut already shows a constriction separating it from the cloaca. The lines along the dorsal margin of the spinal cord represent the boundaries of the myotomes.
- FIG. 32. Embryo No. 371, 6.6 mm., enlarged 31.5 diameters. The segmental levels in this specimen are determined by the sclerotomes. It will be noted that the tail has attained nearly its maximum development, and as compared with the more cranial parts it will hereafter gradually take on a more atrophic appearance.
- FIG. 33. Embryo No. 389, 8 mm., enlarged 31.5 diameters. The coccygeal portion of the spinal cord is already distinctly narrower than the main cord.
- FIG. 34. Embryo No. 544, 11 mm., enlarged 31.5 diameters. In this specimen the caudal gut has disappeared. The vertebrated and non-vertebrated portions of the tail are clearly demarcated.
- FIG. 35. Embryo No. 852, 12 mm., enlarged 31.5 diameters. The non-vertebrated portion of the tail is here relatively much shorter than in the previous specimen.
- FIG. 36. Embryo No. 390, 15.5 mm., enlarged 31.5 diameters. A rudiment of the tail persists as a small elevation (*rud. cau.*). The caudal end of the vertebral column terminates in a fibrous strand of cells. The terminal three sclerotomes may be regarded as having been converted into this strand, or they may have fused into one irregular vertebra.

PLATE 3.

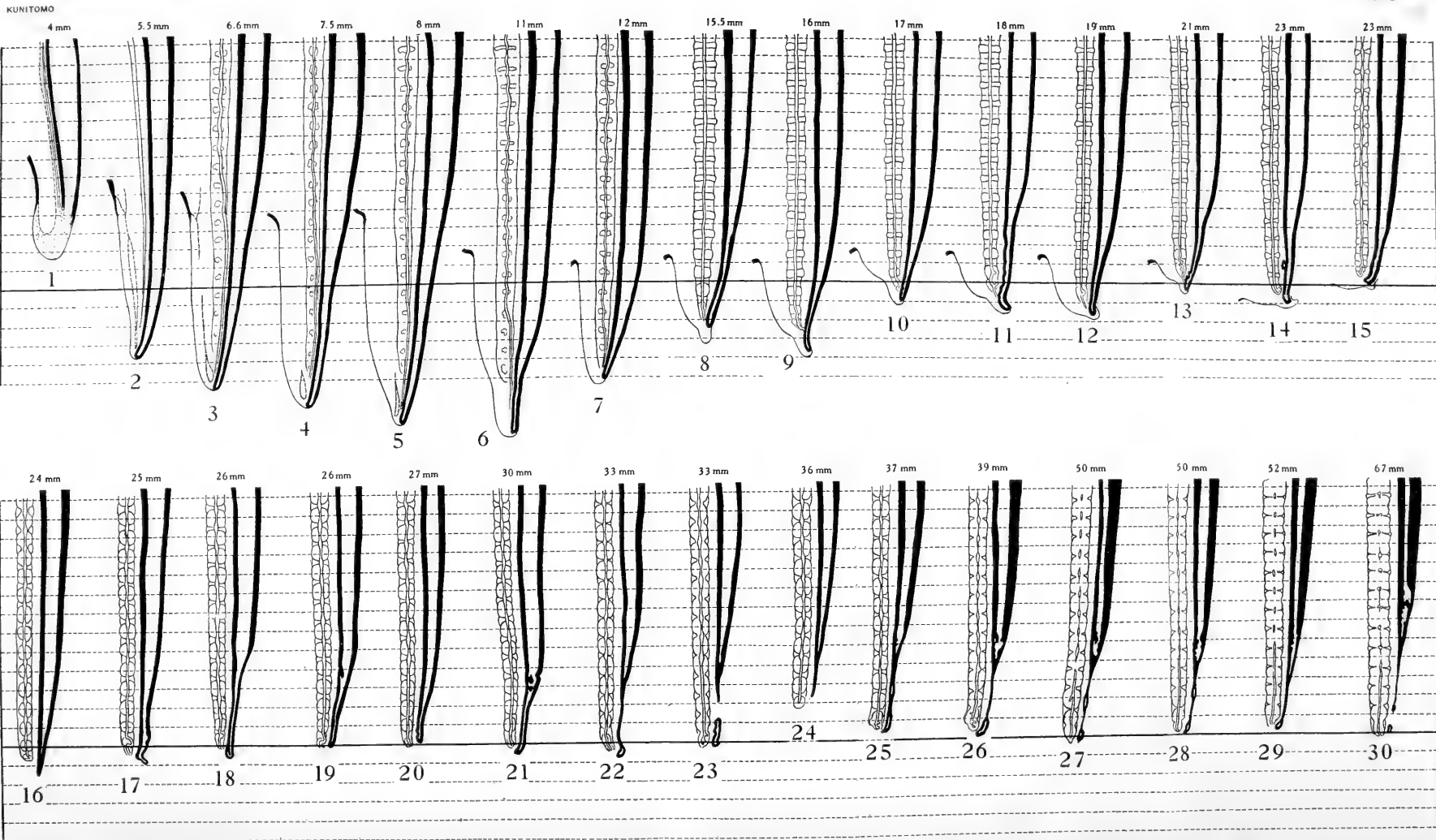
- FIG. 37. Embryo No. 406, 16 mm. crown-rump length, enlarged 31.5 diameters. The slender atrophic portion of the spinal cord is clearly demarcated from the remainder of the cord owing to the fact that it retains its earlier embryonic form. The point at which its narrow canal opens into the main canal corresponds to the future terminal ventricle.
- FIG. 38. Embryo No. 576, 17 mm. crown-rump length, enlarged 22.5 diameters. As compared with the last specimen, the caudal region has undergone marked reduction and resembles the condition that will be seen in embryos about 20 mm. long.
- FIG. 39. Embryo No. 432, 18 mm. crown-rump length, enlarged 22.5 diameters. It will be noted that embryos of about this size show the tendency toward a sharp dorsal retroflexion of the caudal rudiment. The characteristic thinness and wrinkling of the wall of the atrophic portion of the spinal cord is also well represented in this embryo.
- FIG. 40. Embryo No. 453, 23 mm. crown-rump length, enlarged 22.5 diameters. In this specimen, just ventral to the junction of the atrophic part with the remainder of the cord, is a mass of cells which appeared to form a diverticulum, although a communication between its lumen and the central canal of the cord could not be clearly made out.
- FIG. 41. Embryo 382, 23 mm. crown-rump length, enlarged 22.5 diameters. The relative narrowness of the lumen of the atrophic portion of the spinal cord is a characteristic preliminary to its transition into the filum terminale. Near the caudal tip, at the point marked x, is the seat of fusion with the chorda dorsalis.
- FIG. 42. Embryo No. 584, 25 mm. crown-rump length, enlarged 22.5 diameters. A point of fusion with the chorda dorsalis, marked x, can be seen in this specimen somewhat similar to that in figure 41.

PLATE 4.

- FIG. 43. Embryo No. 75, 30 mm. crown-rump length, enlarged 22.5 diameters. The relative thinning-out of the walls of the conus medullaris results in a tendency to their being thrown into large irregular folds. In the region of the conus medullaris the regressive tendency sets in after that region has attained proportions larger than those seen in the more caudal part. It consequently becomes expressed by a thinness of the walls, producing a transparent terminal ventricle in contrast to the obliteration seen in the filum terminale.
- FIG. 44. Embryo No. 972, 37 mm. crown-rump length, enlarged 18 diameters. This specimen shows the transition of the atrophic spinal cord into a fibrous filum terminale. The terminal portion retains its lumen and persists as the coccygeal medullary vestige.
- FIG. 45. Embryo No. 448, 52 mm. crown-rump length, enlarged 13.5 diameters. At this time the terminal ventricle, the filum terminale, and the coccygeal medullary vestige are distinctly marked off from each other, and their general adult characteristics attained.
- FIG. 46. Embryo No. 1656, 67 mm. crown-rump length, enlarged 9 diameters. The regressive condition of the walls of the terminal ventricle are expressed by their relative thinness and their irregularity. The filum terminale is almost entirely converted into a solid fibrous strand in which traces of ependymal masses can be found. The membranes of cord can be seen and present an arrangement that closely simulates that of the adult.

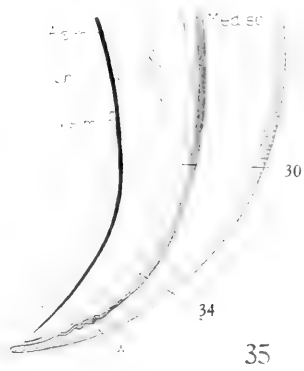
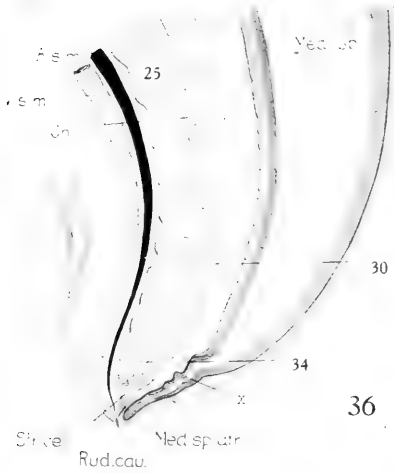
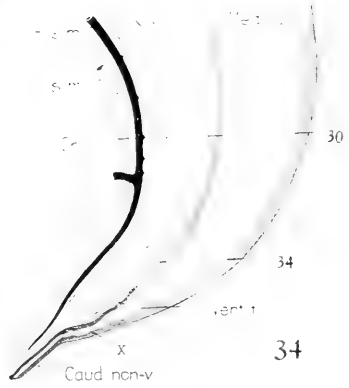
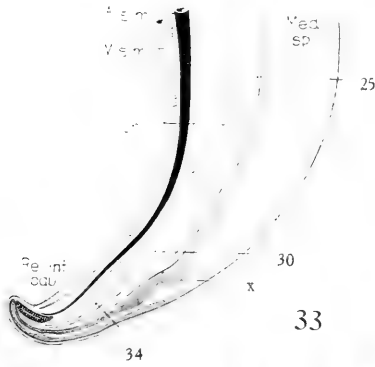
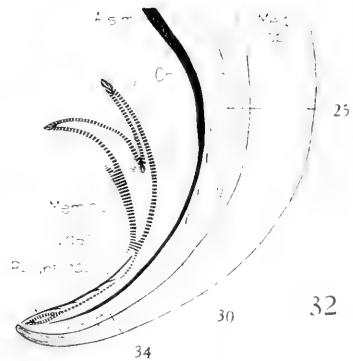
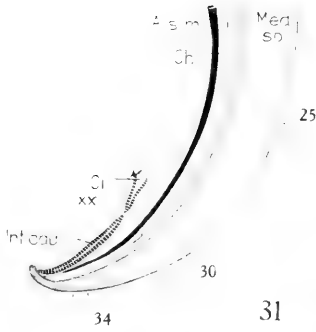


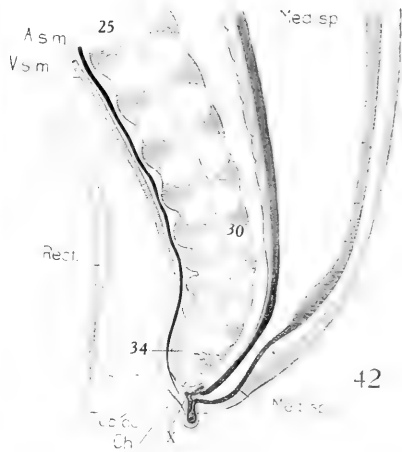
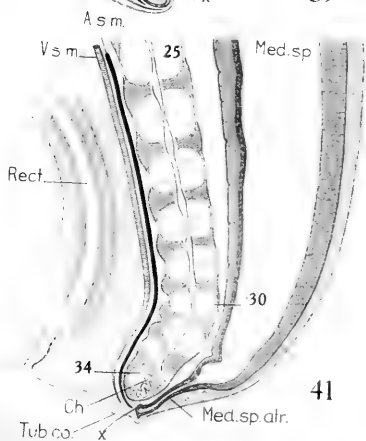
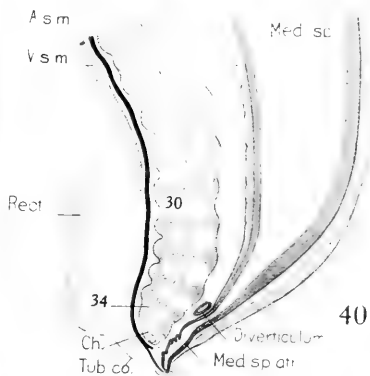
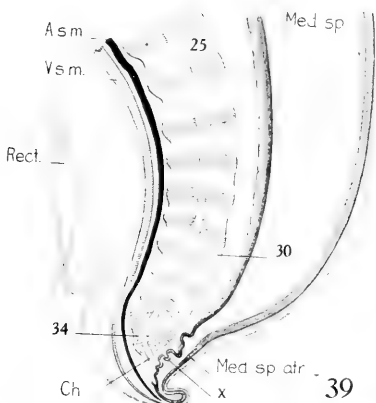
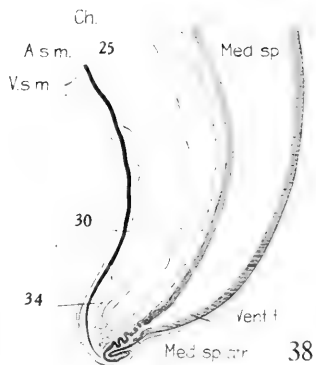
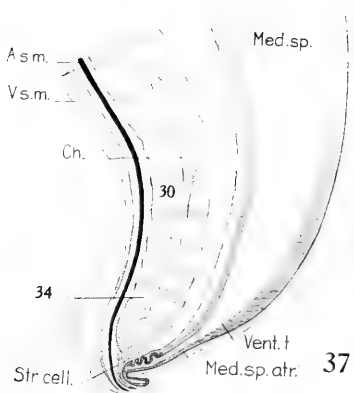
The figure	Fig.	Embryo.
colu	21	No. 75
indi	22	145
stag	23	211
vert	24	449
cau	25	972
diag	26	362
27	27	95
stud	28	184
sho	29	448
res	30	1656

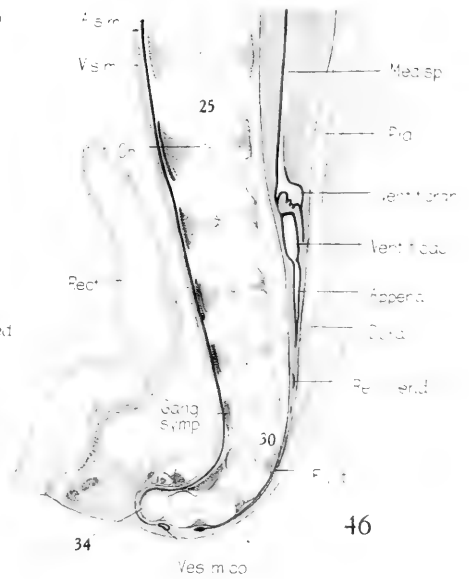
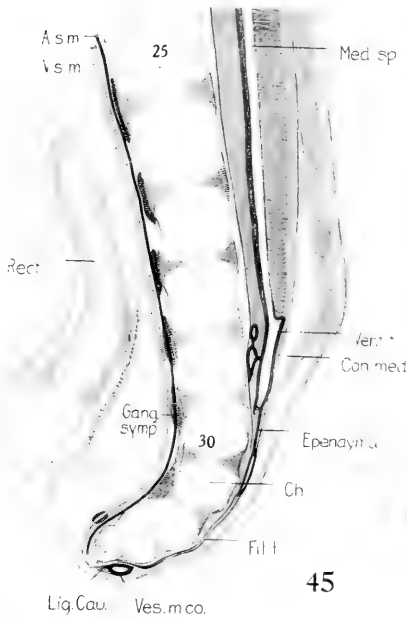
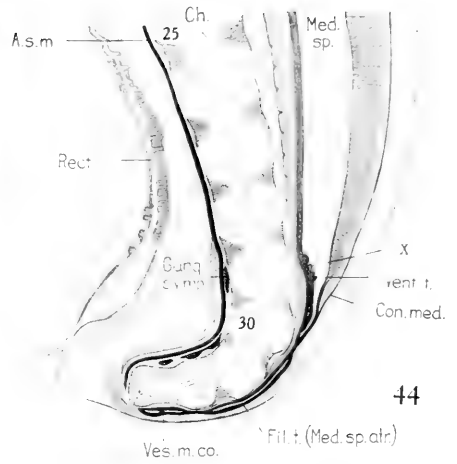
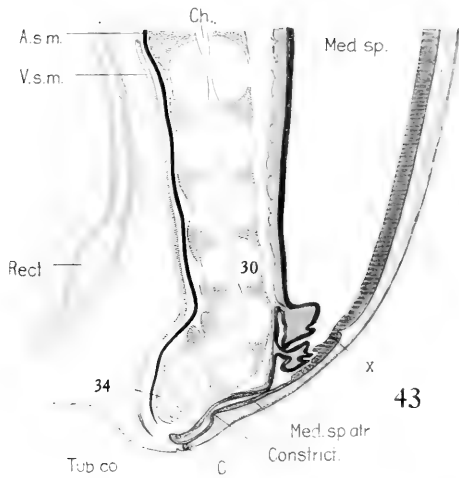


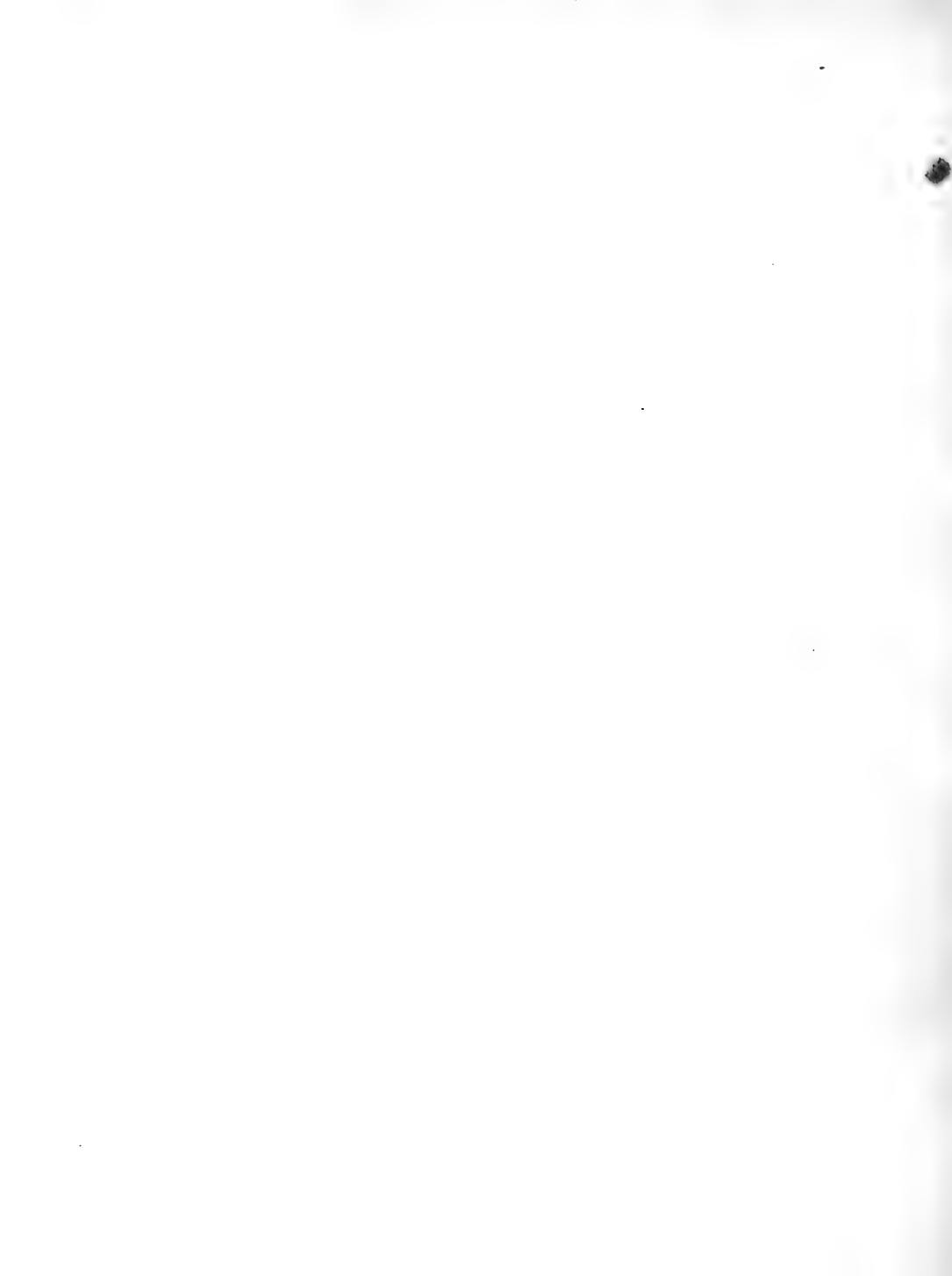
The figures on Plate I are designed to show diagrammatically the relations of the caudal end of the spinal cord and the vertebral column in a series of human embryos varying from 4 mm. to 67 mm. long, and are so arranged that the segmental levels, as indicated at the left, correspond throughout, the thirty-fourth segment being emphasized by a heavy line. In the two younger stages the segments were determined by the myotomes; the remainder were determined by the sclerotomes, or the bodies of the vertebrae. In making the diagram it was found necessary to make all the segments of the same width; in reality the more caudal ones are relatively much narrower. Also, the individual segments become wider in the older stages, whereas in the diagram they are kept at the same width. There is thus introduced an axial distortion which should be kept in mind in studying the figures. In figures 1 to 15 the surface profile of the caudal region is indicated and the cloacal membrane is shown by a wider line. In figures 1 to 5 the caudal gut is shown by a broken line. In figures 4 and 5 it will be noted that a remnant of the gut is still present, though its communication with the cloaca is interrupted. Early stages in the formation of the filum terminale are shown in figures 25 to 30. The figures in this plate are all based upon profile reconstructions made from the following embryos:

Fig.	Embryo.	Fig.	Embryo.	Fig.	Embryo.
1	No. 836	11	No. 432	21	No. 75
2	810	12	837	22	145
3	371	13	431	23	211
4	221	14	453	24	449
5	389	15	382	25	372
6	544	16	632	26	362
7	852	17	584a	27	95
8	390	18	405	28	184
9	406	19	1008	29	448
10	576	20	875	30	1656











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