CONTRIBUTIONS TO EMBRYOLOGY

VOLUME VIII, Nos. 24, 25, 26

CONTENTS.

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.

CONTENTS.

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 endo-thelium 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, con-forming 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 stratifi-cation 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 sur-rounding 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 arrange-ment 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, 1917a, 1917b). 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, 1917b), 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-corpuscles. 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 (1917a), 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 (1917a).

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 (1917a) 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 (1917a). 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 (1917a) 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 drainge-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 (1917a). 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 (1917a) 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 facial 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 stapedial 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
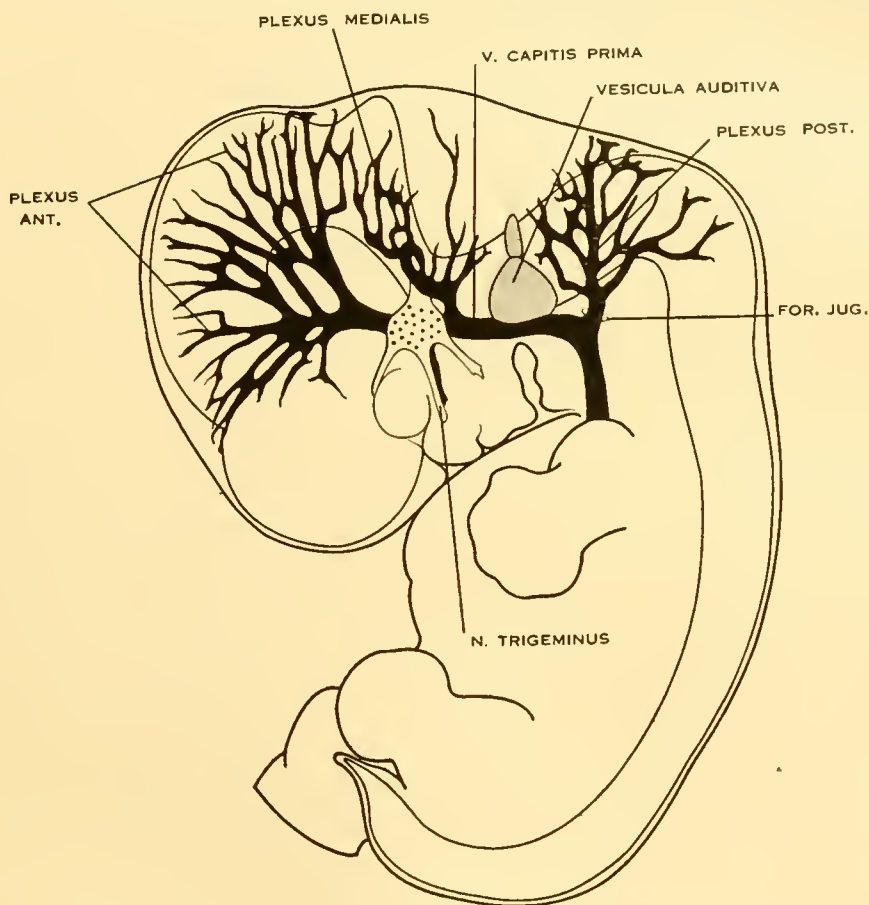

FIGURE 1.

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 (1917a) 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.
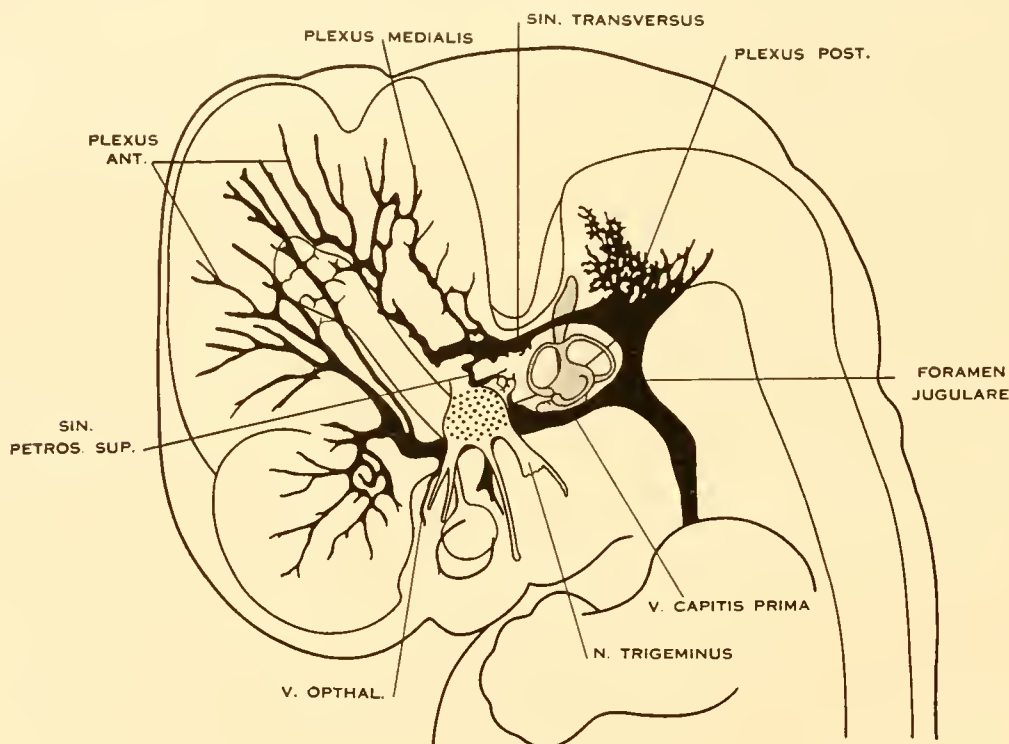

FIGURE 2.

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 intercranial (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
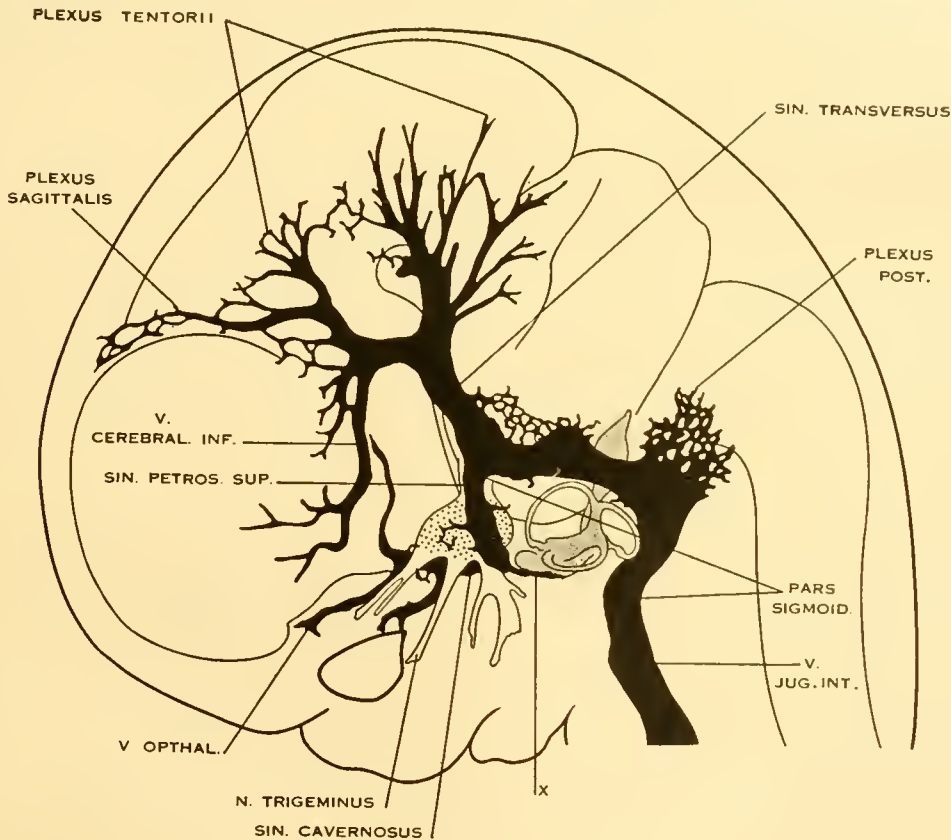

FIGURE 3.

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 cerebralis 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 character-
istic 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 differ-
entiated along the margin of the cerebrum and between the hemispheres a sub-
division of this plexus that is eventually to constitute the superior sagittal sinus
(marked plexus sagittalis in figs. 3, 4, and 26).
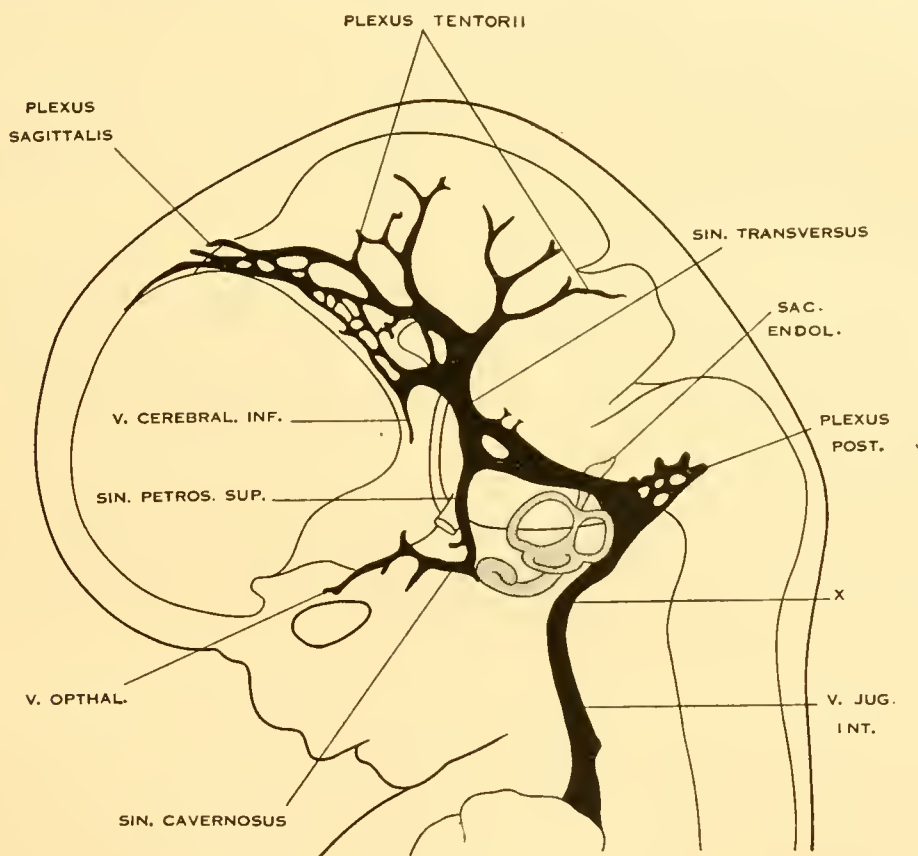
FIGURE 4.

Profile reconstruction of the main dural veins in a human embryo 24 mm. long (Carnegie Col-
     lection, 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.
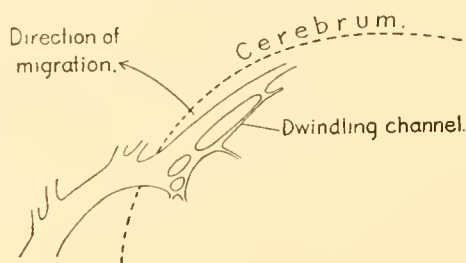
the other hand, a vein may change its position by forming or adopting a new endothelial channel and at the same time relinquishing its original endothelial channel. The embryonic plexiform character of the veins in the region of the tentorium is especially favorable for this procedure, and we find this 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 carti-laginous 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 caudal-ward 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
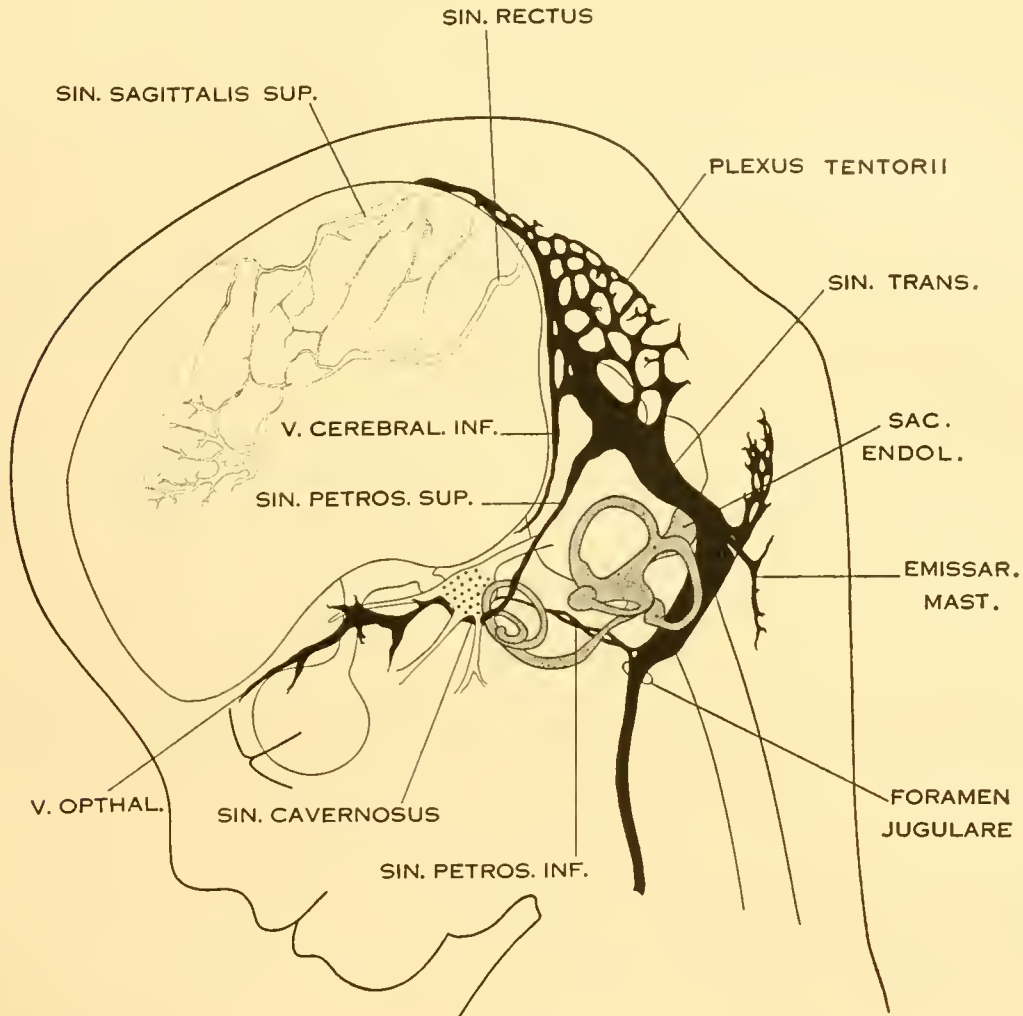
FIGURE 6.

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 mesh-work, 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 adjust-mental 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 em-bryos 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 longi-tudinal 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
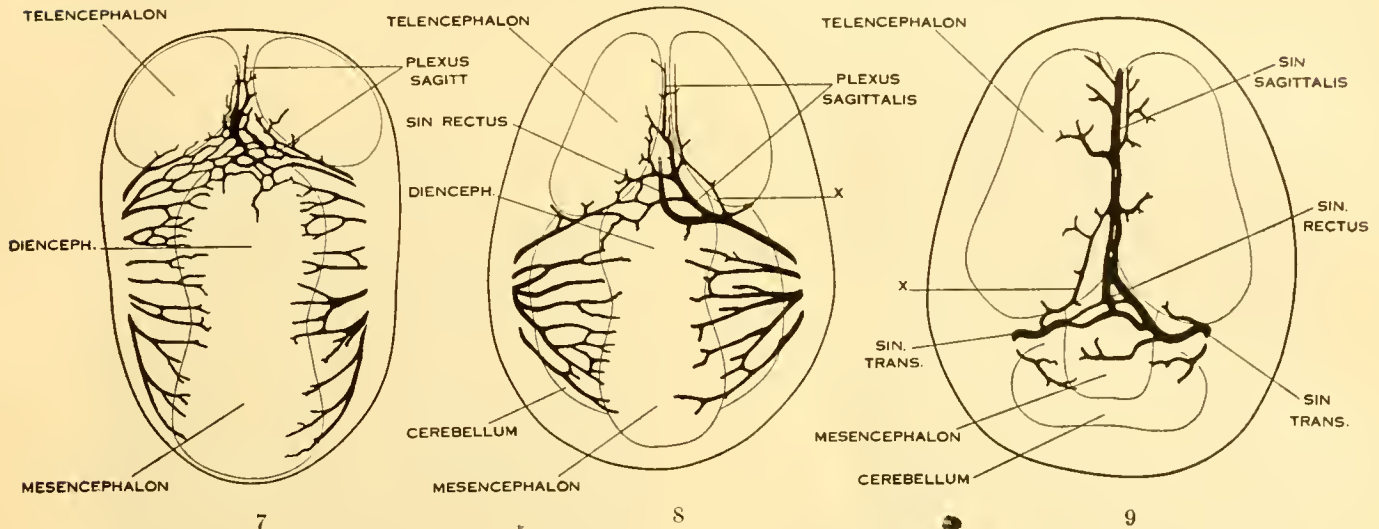
Figs. 7, 8, and 9.—Three stages in the formation of the sagittal plexus, showing its asymmetrical character and its conversion into the superior sagittal sinus. Draining into it from below is the drainage-channel from the chorioidal bodies that is later known as the straight sinus. The channels marked 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. 458) 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. 15b;

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.
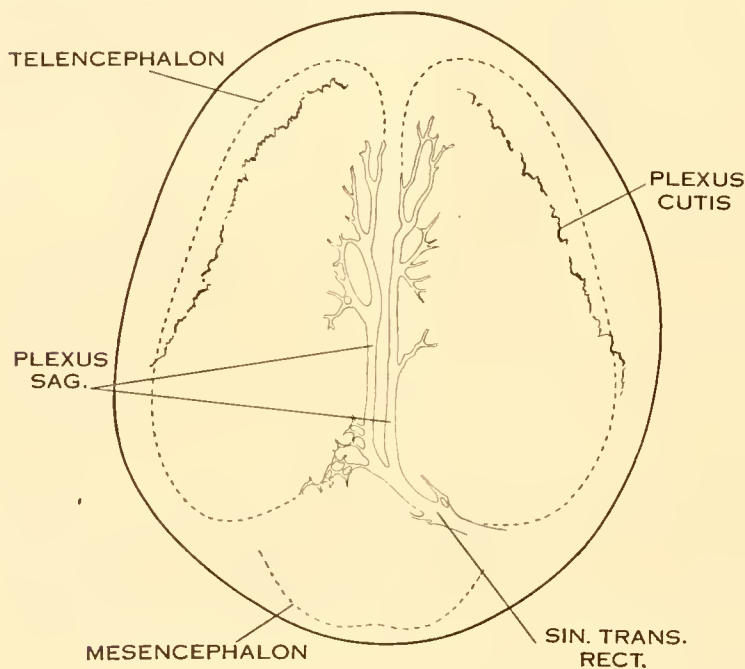

FIGURE 10.

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.

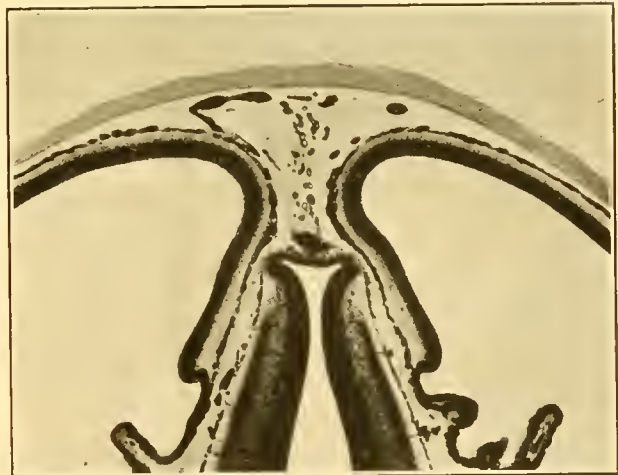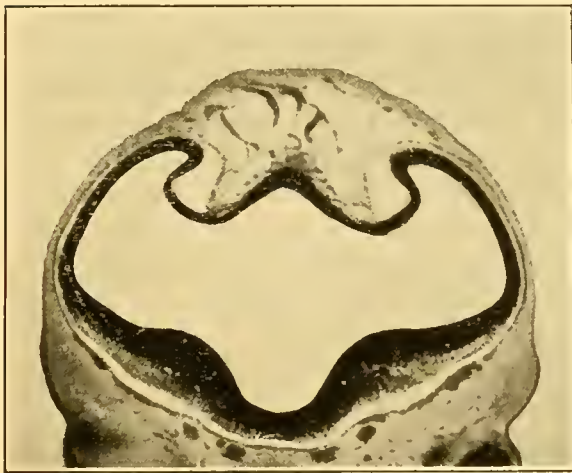11   12

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.
	mm.			*mm.*	
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.

REFERENCES CITED.

ELZE, C. 1907. Beschreibung eines menschlichen Embryo von za. 7 mm. grösster Länge. Anat. Hefte, No. 106, vol. 35, Abth. 1.

EVANS, H. M. 1909. On the development of the aortæ, cardinal and umbilical veins, and other blood-vessels of vertebrate embryos from capillaries. Anat. Rec., vol. 3.

————. 1912. The development of the vascular system. Keibel-Mall Manual of Human Embryology, vol. 2.

GROSSER, O. 1907. Die Elemente des Kopfvenensystem der Wirbeltiere. Verh. Anat. Gesell., Anat. Anz., Bd. 30, Ergänz. Hft.

———— und E. BREZINA. 1895. Ueber die Entwicklung der Venen des Kopfes und Halses bei Reptilien. Morph. Jahrbuch, Bd. 23.

HIS, W. 1904. Die Entwickelung des menschlichen Gehirns. Leipzig.

HOCHSTETTER, F. 1916. Ueber die Vaskularisation der Haut des Schadeldaches menschlicher Embryonen. K. Akad. d. Wiss. Wien, Math.-Naturwiss. Kl., Bd. 93.

INGALLS, N. W. 1907. Beschreibung eines menschlichen Embryo von 4.9 mm. Archiv. f. mikr. Anat., vol. 70.

MALL, F. P. 1905. On the development of the blood-vessels of the brain in the human embryo. Am. Jour. Anat., vol. 4.

MARKOWSKI, J. 1911. Ueber die Entwicklung der Sinus durae matris und der Hirnvenen bei menschlichen Embryonen. Bull. d. l'Acad. d. Sci. de Cracovie. Cl. d. Sci. math.-Nat., Serie B.

SABIN, F. R. 1915. On the fate of the post-cardinal veins and their relation to the development of the vena cava and azygos in the embryo pig. Contributions to Embryology, Carnegie Inst. Wash. Pub. No. 223.

————. 1917a. Origin and development of the primitive vessels of the chick and of the pig. Contributions to Embryology, Carnegie Inst. Wash. Pub. No. 226.

————. 1917b. Preliminary note on the differentiation of angioblasts and the method by which they produce blood-vessels, blood-plasma, and red blood-cells as seen in the living chick. Anat. Rec., vol. 13.

SALZER, H. 1895. Ueber die Entwicklung der Kopfvenen des Meerschweinchens. Morph. Jahrbuch, Bd. 23.

SHINDO, T. 1915. Ueber die Bedeutung des Sinus cavernosus der Säuger mit vergleichend anatomischer Berücksichtigung anderer Kopfvenen. Anat. Hefte, Abth. 1, Heft 157, Bd. 52.

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.
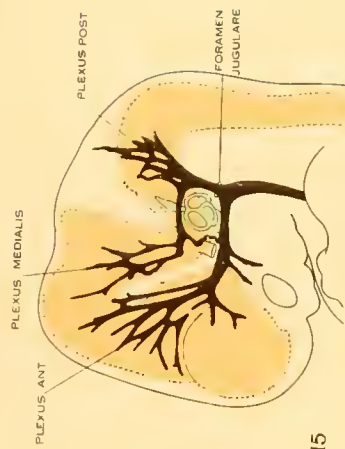
Plate 3.

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.

PLATE 1

PLATE 2

A pulmon.

V card post

5th seg art

V umbl s

Plexus post

Vesicula auditiva

Plexus medialis

N. trigem

V capitis prima

Bulbus

Atr. s

A. mes. sup

12th seg art

Plexus ant

Ventr

A. mes inf

A coud sin

A umbil s

V card post

Plexus ont

Fig. 23

Plexus med

Plexus ant

A. car. int

A. caud. dext

V. capitis prima

A. umbil. dext

Plexus post

Bulbus

Atr dext

A. mes. inf.

V. card. post.

V. umbil. dext.

V card. mes.

V. omph-mes dext

Fig. 22

PLATE 3

Foramen jug
A.vert
A.cor ext
A.subclav.
V.subclav.
V.cav inf
V.card post

Plexus post

V.capitis prima
A.car int

A.coelioca
A.mes. sup.
A.mes inf

Plexus medialis

A.cerebri med
Plexus max
A.umbilicalis
V.opthal
A.sacr med

Fig. 25

Plexus ant.
A basilaris
Plexus ant.
N.trigem
Capillary sheet of brain wall
Plexus maxillaris

A basilaris
Plexus medialis
Vesicula auditiva
Plexus post
A vert

V.capitis prima

Fig. 24

PLATE 4

A. cerebri post

Plexus sagittalis

Sin.rectus

A. chorioidealis

Plexus chorioideus

A. cerebri ant

A et V cerebri med

V. opthal

A.centralis retinae

Plexus max

A.max int.

A.max ext

A.lingualis

A.carotis int.

Sin covern.

Sinus transversus

Plexus tentorii

A.cerebelli sup.

Plexus post

Sin petros. sup.

A.occip

V.jug int

A.vert

Fig. 26

PLATE 5

Plexus Sagittalis

Plexus tentorii

Sin. transversus

S petr sup

Emissar mast

Sin cav

V opthal

Bulbus V jug. sup

A car int

4 cerebri med

V fac com

A car ext

A vert

V jug int

A car com sin

A subclav

A anonyma

A pulmon

Fig. 27

CONTRIBUTIONS TO EMBRYOLOGY, No. 25.

THE MITOCHONDRIAL CONSTITUENTS OF PROTOPLASM

By E. V. Cowdry.

One plate and nine text-figures.

CONTENTS.

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 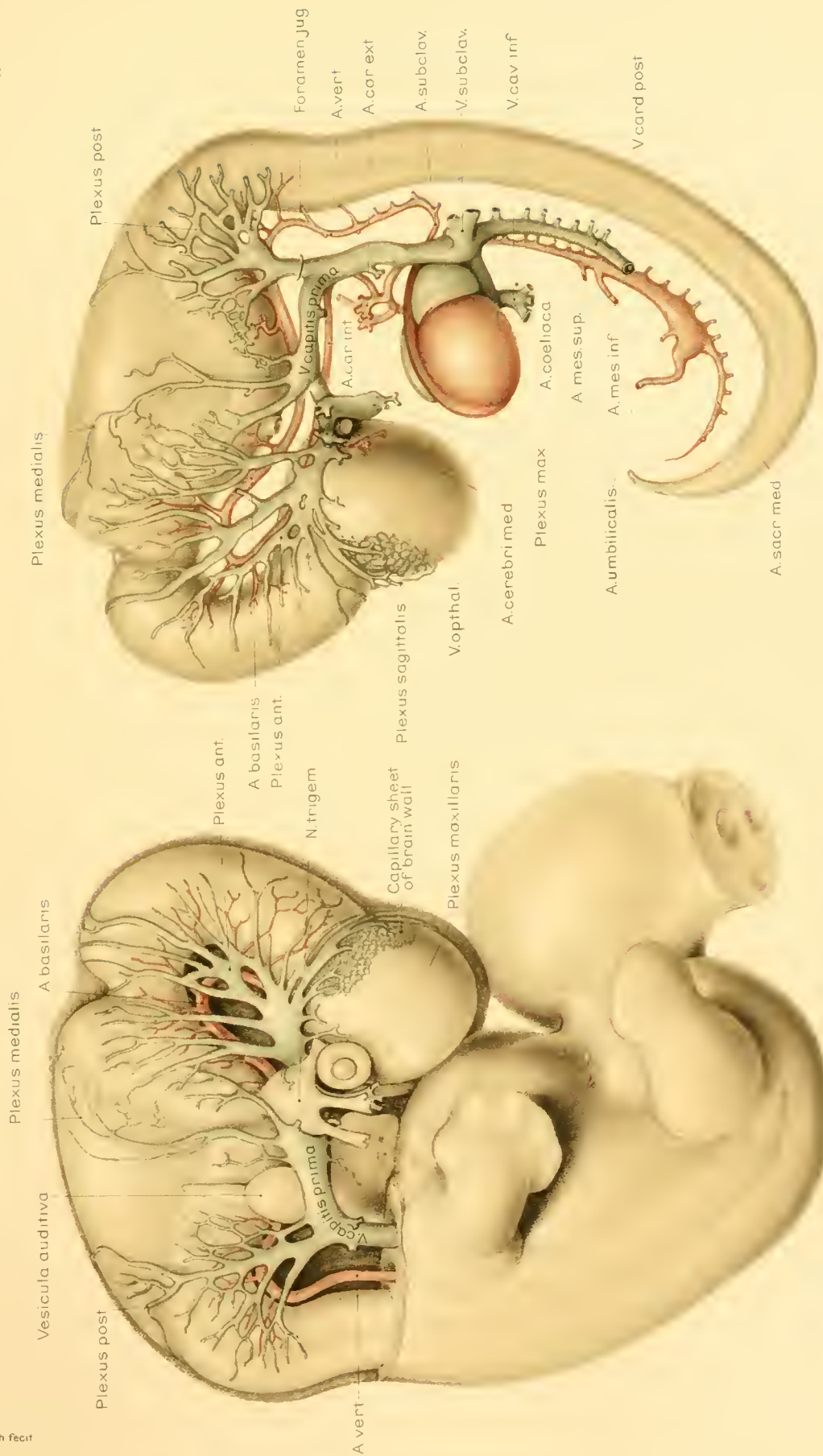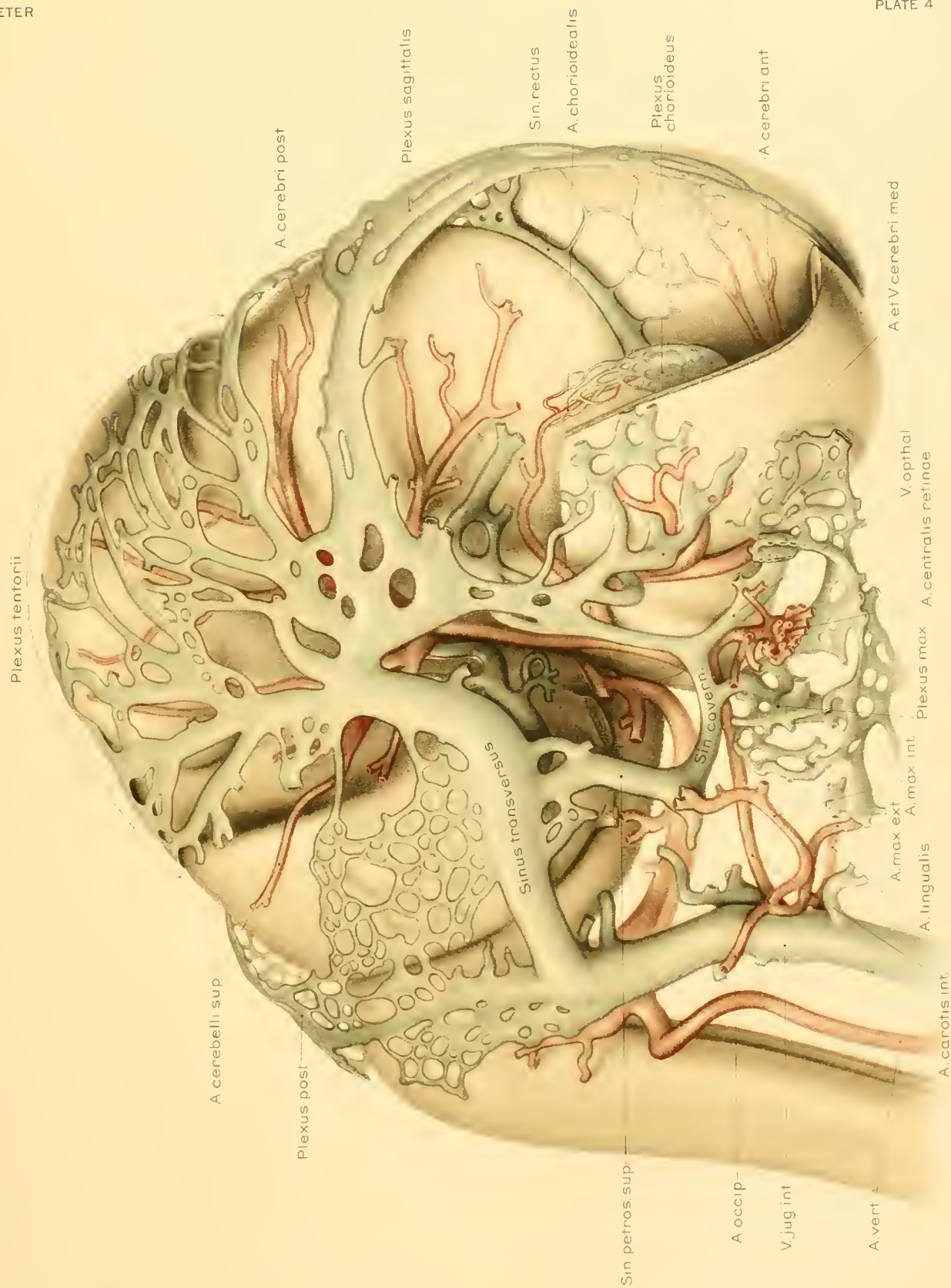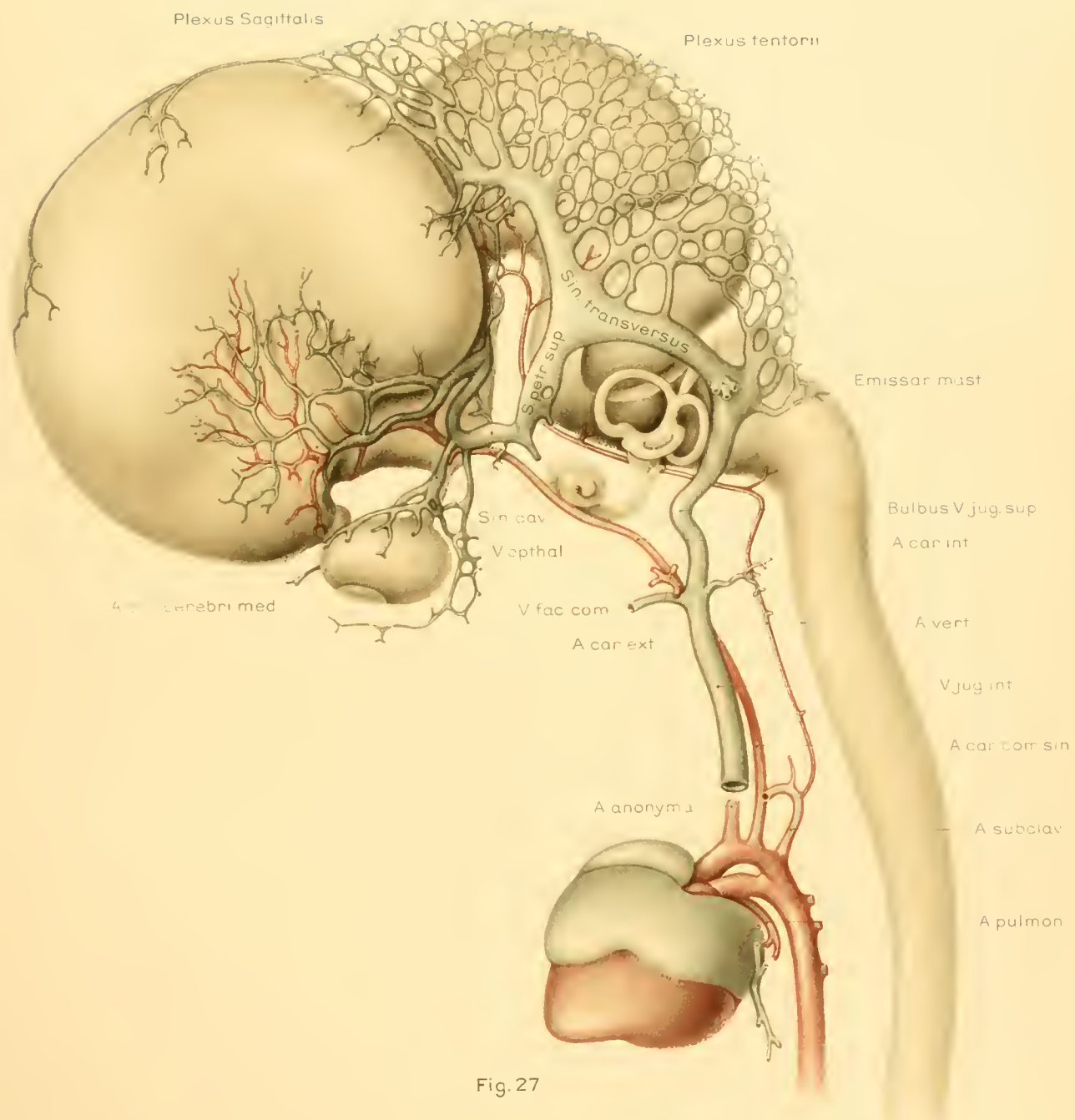of bacteria), their lipoid properties, and their extraordinary sensitivity to certain types of pathological change have become familiar. These and other discoveries have stimulated interest in many quarters and have given rise to hastily conceived and poorly supported theories and to much discussion, 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 mitochondra 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

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 fucsinofili" 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 (1899a, 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 (1908d, 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; 1913b, 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)	(ἄρχων, 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 the same as the apparato reticolare interno.
Caryosomochondria	Arndt (1914, p. 55)	By definition mitochondria arising from the caryosome.
Caudal 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 1—*Continued.*

Term.	Author.	Remarks.
Chondriom	Meves (1907a, p. 403)	Cytoplasmic content of mitochondria.
Chondriomiten	Benda (1899a, p. 382)	Thread-like mitochondria.
Chondriomitom	Benda (1898, p. 397)	A feltwork of mitochondria.
Chondrioplastes	Champy (1913a, p. 157)	By definition plast-forming mitochondria.
Chondriorhäbden	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 apparato reticolare interno.
Cytomicrosomes	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).
Enchylemma	Butschli	More fluid part of protoplasm (Conklin, 1917, p. 357), contains mitochondria.
Endoplasm		(1) Deeper protoplasm often said to be nutritive by contrast (Renaut 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 juxtanucleaires		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, Dnesberg (1910, p. 653); (2) same as nucleus of Balbiani (Sjovall, 1906, p. 577). Should not be confused with "idiosome," the hypothetical carrier of heredity.
Interstitialkörner	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 chondriomitom, Prenant (1899, p. 429); (2) more solid part of protoplasm, Conklin (1917, p. 357).
Körnern	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	($\mu\iota\kappa\rho\acute{o}s$, small, and $\sigma\tilde{\omega}\mu\alpha$, a body.) A most general term, including of course mitochondria.
Mitochondria	Benda (1898, p. 397; 1899a, p. 382).	($\mu\acute{\iota}\tau os$, a thread, and $\chi\acute{o}\nu\delta\rho os$, a grain.) Fadenkornern, thread granules.

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

TABLE 1—*Continued.*

Term.	Author.	Remarks.
Mitochondrial apparat	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 chromidial substance (chromophile and tigroid substances, etc.), but quite distinct from mitochondria (Cowdry, 1911, p. 753; 1913b, p. 311).
Parabasal bodies		These, according to Alexeieff (1917b, p. 501), are homologous with mitochondria.
Paramitome	Flemming (1882, p. 77)	(1) Same as paraplasm (Fauré-Fremiet, 1912a, p. 408); (2) the more fluid part of protoplasm (Conklin, 1917, p. 357).
Paranucleus		Same as chondriomitom (Schäffer, 1912, p. 24).
Péricaryonème (périnème)	Renaut and Dubreuil (1906b, p. 230).	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	Wiesner	($\pi\lambda\acute{a}\sigma\mu a$, a thing formed, and $\sigma\bar{\omega}\mu a$, a body.) Ultimate vital units not to be confused with plastosomes.
Plastidulen	Maggi	Probably in part mitochondria.
Plastochondrien	Meves (1910a, p. 150)	Granular mitochondria playing a designated part in histogenesis.
Plastochondriomiten	Meves (1910a, p. 150)	Rows of granules.
Plastoconten	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.
Saftkanälchen	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).
Spiremes	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.
Trophospongium	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.
Vermicules	Laguesse (1900, p. 5)	Certain filamentous mitochondria present in gland cells.
Vibrioides	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 (1899a, p. 397), who introduced it to designate certain "Fädenkörnern" which he had been studying in spermatogenesis. It is derived from the Greek μίτος, a thread, and χόνδρος, 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 (1907a, 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), "Chondriorhäbden" (shaft-like forms), "Chondriosphären" (spheres), and "Chondriom" or "Mitochondriom" (the cytoplasmic content of mitochondria of whatever form).

The words "chondriorhäbden" 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); "chondriocontes" (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, 1916a, 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.

---

[1] In 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, chondriocontes, 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.
Chondriocontes	Rods	Filaments	Not used	Filaments.
Chondriomites	Filaments of grains	Not used	Homogeneous filament	Not used.
Chondriosomes	More or less voluminous body, larger than preceding, 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 (1910a, 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 "Plastoconten"). 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 "plasmasome," 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 (1913a, 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 1899a (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 (1916a 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 (1899a, p. 382), who refers to them continually as "*Faden*körnern." The term "mitochondrium" is the Latin singular of the Greek compound word. The correct Greek singular is mitochondrion.[1]

[1] I 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 zo 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 chondriocontes), 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 have 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 Mawas (1909b, 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, Guieysse-Pélissier (1910, p. 18).

Alimentary tract—*continued.*
  Liver, Altmann (1894, p. 155).
    Bile ducts, Policard (1914, p. 623).
Respiratory tract:
  Bowman's glands, ———.
  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); Policard (1915, p. 539).
  Pronephros and mesonephros in amphibia, Luna (1913f, p. 131).
    Bladder, ———.
    Urethra, ———.
    Glands of Littré, ———.
    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).*
    Prepucial glands (glands of Tyson), Altmann (1890, p. 99).
    Corpus cavernosum, ———.
    Corpus spongiosum, ———.
    Ovary, Zoja brothers (1891, p. 259), under head of "Plastidulen."
    Insterstitial 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).
    Coccygeal gland, Altmann (1890, p. 145).

ANIMALS—*Continued.*

Connective tissue:
 Fibroblasts, Dubreuil (1913, p. 95).
 Clasmatocytes, Dubreuil (1913, p. 87).
 Plasma cells, *Schridde (1905, p. 729)*; Dubreuil (1909,
  p. 80); and *Dubreuil and Favre (1914b, p. 24).*
 Mast cells (basophile 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 (myeloplaxes, 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, Altmann (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, ———.

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, *Alagna (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, Lewises (1914, p. 330); Levi
  (1916a, p. 101); Maximow (1916b, p. 465).

PLANTS.

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 hyphæ, 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).
  Teleutospore, Puccinia, Beauverie (1914b, p. 359);
   Phragmidium, Mme. Moreau (1914, p. 421).

Reproductive system—*continued.*
 Thallophytes—*continued.*
  Uredospore, Coleosporium, Mme. Moreau (1914,
   p. 421).
  Æcidospore, Phragmidium, Mme. Moreau (1914,
   p. 421).
 Bryophytes and pteridophytes:
  Spore, sporogenous-tissue cells, tapetum, spore-
   mother cells; Funaria, Sapehin (1915, pl. 22);
   Anthoceras, Scherrer (1913, pl. 20).
  Sperm, spermatogenous-tissue cells, sperm mother-
   cells, antheridium mother-cells; Polytrichium,
   Funaria, Bryum, Marchantia, Sapehin (1915,
   pls. 22–26); Anthoceras, Scherrer (1913, pl. 20).
  Egg, ventral-canal cell, neck-cells of archegonium;
   Funaria, Bryum, Sapehin (1915, pl. 26); Antho-
   ceras, Scherrer (1914, pl. 20).

PLANTS—*Continued.*

Reproductive system—*continued.*

Spermatophytes:

Pollen, pollen mother-cells, tapetum; Nymphæa, Meves (1904, p. 284); Asparagus, Lewitsky (1910, pl. 17); Lilium, Cucurbita, Guilliermond (1912a, fig. 6; 1914b, fig. 6).

Embryo sac, ovule, antipodals, synergids, embryo tegument, nutritive cells; Lilium, Guilliermond (1912a, figs. 4, 5; 1914b, fig. 13); Pensa (1911, fig. 2).

Seed, barley, Ricinus, Guilliermond (1912a, fig. 7).

Vegetative system:

Filaments of algæ and fungi; Vaucheria, Achlya, Rudolph (1912, pl. 18); Penicillium, Endomyces, Guilliermond (1914b, fig. 10).

Apical cells of algæ, 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.

18); Lewitsky (1910, pl. 17); Tropæolum, Guilliermond (1912a, fig. 2); Tulip, Pensa (1911, figs. 6, 7);

Secondary meristem, cambium, Guilliermond (1912a, p. 358).

Epidermis of stem, root, leaf, flower, fruit; Asparagus, Trianea, Sapehin (1915, pls. 10, 11); Chenopodium, Juglans, Iris, Tulip, Clivia, Tropæolum, Guilliermond (1917, p. 233; 1914b, fig. 14).

Mesophyll cells, palisade cells, leaves, carpels; Nereum, "haricot," Guilliermond (1914b, fig. 11, 12; 1912a, pl. 18).

Leaf cells, stem-tip of mosses, Polytrichium, 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, Guilliermond (1914a, p. 566).

Hair cells, Cucurbita, Maximow (1913, p. 243).

Giant cells of Heterodera galls; Vitis, Němec (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 paramœcium, Prowazek, according to Fauré-Fremiet (1910a, p. 510).

Trypanosoma lewisi, Shipley (1916, p. 444).

Blastocystis enterocola, Trichomonas angusta, Alexeieff (1916b, p. 1074).

Giardia cunicula, anadenalis, muris, and agilis, Trichomonas batrachiorum and muris, Hexamastix termitis, Octomastix parva and motellæ, Octomitus intestinalis, Trichonympha agilis, Entrichomastix motellæ, and Tetramastix, Alexeieff (1917b, p. 499).

Protozoa—*continued.*

Sporozoa:

Monocystis ascidiæ, Hirschler (1914, p. 304).

Hæmogregarina sergentium, Viguier and Weber (1912, p. 92).

Infusoria:

Vorticella, under heading of "Spheroblasts," by Czermak, 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 polypinum, Spirostomum ambiguum, Fauré-Fremiet (1910a, p. 504).

Campanella ——, Fauré-Fremiet (1910a, p. 506).

Paramœcium 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).
  Cyttarrocyclis Ehrenbergii, under heading of "Chromidia," Gesa Entz, jr., according to Fauré-Fremiet (1910a, p. 514).
  Tintinnidium inguilinum, Fauré-Fremiet (1910a, p. 514).
  Opisthonecta henneguyi, Fauré-Fremiet (1910a, p. 516).
  Trichodinopsis paradoxa, Fauré-Fremiet (1910a, p. 517).
  Opercularia racemosa, Fauré-Fremiet (1910a, p. 519).
  Opercularia notonectæ, Fauré-Fremiet (1910a, p. 520).
  Noctiluca miliaris, Fauré-Fremiet (1910a, p. 524).
  Opalina ranarum, Colopoda cucullus, Stentor cucullus and polymorphus, Isotherica prostoma, Entodinium bursa and minimus, Diplodinium maggii and cattanei, Butschlia parva, L. and R. Zoja (1891, pp. 243, 244).
  Blantidium enterozoon, 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," Pictet, according to Fauré-Fremiet (1910a, p. 571).
  Hydra vulgaris, L. and R. Zoja (1891, p. 244).
 Scyphozoa:
  Aurelia aurita, Tsukaguchi (1914, p. 117).
 Actinizoa: ———.
 Ctenophora: ———.
Platyhelminthes:
 Turbellaria:
  Plagiostoma girardi, spermatids, Weygandt (1907, p. 271).
  Planaria adherens, almost all cells of the body, Korotneff (1909, p. 1010).
  Trematoda:
  Dicrocœlium lanceatum, spermatogenesis, Dingler (1910, p. 698).
  Cestoda: ———.
  Nemertinea: ———.
Nemathelminthes:
 Nematoda:
  Ascaris lumbricoides, male and female germ-cells, Hirschler (1913, p. 353).
  Ascaris megalocephala, gonocytes, Fauré-Fremiet (1912b, p. 346); L. and R. Zoja (1891, p. 245).
  Filaria papillosa, Meves (1915b, p. 38).
  Acanthocephala: ———.
  Chætognatha: ———.
Trochelminthes:
 Rotifera: ———.
 Dinophilea: ———.
 Gastrotricha: ———.
Molluscoida:
 Polyzoa:
  Membranifera pilosa L., spermatogenesis, Bonnevie (1907, p. 578).

Molluscoida—*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, Sphærechinus granularis, Spatangus purpureus, under heading of "Cytomicrosomes," in spermatogenesis, Pictet, 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:
 Chætopoda:
  Nereis virens, nerve-cells, Cowdry (1914a, p. 11).
  Serpula uncinata, Nais proboscidæ, L. and R. Zoja (1891, p. 248).
  Tapinambis tegnixin, kidney, Mayer and Rathery (1909, p. 335).
  Lumbricus herculeus and terrestris. Allobophora terrestris, kidney, Maziarski (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 adspersus, 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 Grobben, according to Fauré-Fremiet (1910a, p. 551).
  Maia squamado, Carcinus mœnas, 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, Guieysse-Pélessier (1910, p. 18).
  Pandarus sinuatus, spermatogenesis, McClendon (1910, p. 234).

ANIMALS—*Continued.*

Onychophora: ———.
Myriopoda: ———.
  Pachyiulus 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 carpophagus, Polyxenus lagurus, Polyzonium 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).
  Euscorpius carpathicus L., Buthus eupeus, spermatogenesis, Sokolow (1913, p. 398).
  "Arizona scorpion," Wilson (1916, p. 539).
Insecta:
  Forficula auricularia, Valette St. George (1887), Blatta germanica, Periplaneta, spermatids, Henneguy (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, Giglio-Tos and Granata (1908, p. 115).
  Stenobothrus biguttulus, spermatogenesis, Gerard (1909, p. 599).
  Locusta viridissima, Gryllus campestris, spermatogenesis, Henneguy (1904).
  Gryllus assimilis, spermatid, Baumgartner (1902, p. 50).
  Gryllus campestris, primary spermatocytes, Henneguy (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 jacobeæ, Cossus ligniperda, Bombyx lanestris 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).
  Pygæra bucephala, spermatogenesis, Meves (1900, p. 556).
  Bombyx ———, spermatogenesis, Henneguy (1904).
  Oryctes nasicornis, spermatogenesis, Prowazek.[1]

Insecta—*continued.*
  Silpha carinata, spermatogenesis, N. Holmgren (1902, p. 197).
  Cybister roeselii, 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: ———.
  Gasteropoda:
    Enteroxenes östergeni, spermatogenesis, Bonnevie (1904, p. 269).
    Conus mediterraneus, Vermetus gigas, spermatogenesis, Kuschakewitsch (1911, p. 532).
    Fulgar canaliculatus, nerve-cells, Cowdry (1914a, p. 11).
    Helix pomatia, Acanthopsole 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 Pictet, 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 mentala, Molgula socialis, Cynthia tatraedra, 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 branchialis, nerve-cells, Mawas (1910a, p. 126).
    Myxine glutinosa, primary spermatocytes, Schreiner A. and K. E. (1905, p. 296).

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

Chordata—*continued.*
  Pisces:
    Perca americana, Morone americana, Salmo irideus, Fundulus heteroclitus, embryos, Duesberg (1917, p. 465); Coregonus muræna, germ-cells, Aunap (1913, p. 454); Box salpa, Tinca vulgaris, Corti (1913, p. 52); Anguilla vulgaris, interrenal, in testine, Comolli (1913, p. 408).
    Bream, pike, and chevasson, kidney, Policard and Mawas (1906, p. 218).
    Scyllium canicula, choroid plexus, Grynfeltt and Euziere (1913b, p. 104).
    Salmo ——, eggs, Cermak (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).
    Tinca vulgaris, Hyla arborea, L. and R. Zoja (1891, p. 256).
    Rhacophorus, larvæ, Saguchi (1913, p. 185).
    Geotriton fuscus, pancreas, Levi (1912, p. 579).
  Reptilia:
    Clemmys marmorata, Lacerta muralis, oogonia, 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 græca, nerve-cells, Busacca (1913, p. 327).
    Cistudo europeæ, muscle, Aimé (1911, p. 270).
    Platydactylus 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 atracapilla, Gallus domesticus, Emberyza citrinella, L. and R. Zoja (1891, p. 256).
  Mammalia:
    Erinaceus europeus, intestine, Corti (1913, p. 188).
    Didelphys virginiana, spermatogenesis, Jordan (1911, p. 58).
    Phocæna 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 familaris, 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 schreiberii, ovaries, Levi (1913, p. 518).
    Many apes, spermatogenesis, Moreaux (1909, p. 371).
    Macaccus 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 cobaya, Felis domestica, Homo sapiens, L. and R. Zoja (1891, p. 256).

PLANTS.

Myxomycetes: ——.
Schizophytes: ——.
Algæ:
    Cystoseira barbata, apical cell, Nicolosi-Roncati (1912b, p. 144).
    Fucus serratus, apical cell, Le Touze (1912, plate 9).
    Gastroclonium reflexum, Gigartina teedi, Lemanea torulosa, carpospores and tetraspores, Nicolosi-Roncati (1912a, p. 60 (?).
    Vaucheria, filaments, Rudolph (1912, plate 18).
    Coccomyxa solorinæ, mitochondria absent, the Moreaus (1916, p. 212).
Fungi:
    Achlya, hyphæ, Rudolph (1912, plate 18).
    Albugo bliti and candida, hyphæ and conidia, Lewitsky (1913, pl. 21).
    Aleuria cerea, asci, Guilliermond (1913a, p. 618).
    Botrytis cinerea, Guilliermond (1913g, p. 1781).
    Coleosporium senecionis, Mme. Moreau (1914, p. 421).
    Endomyces magnusi, fibuliger, and albicans, Guilliermond (1913g, p. 1781).
    Entyloma ranuculi, 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 subcorticum, telautospore, Mme. Moreau (1914, p. 421).
    Psalliota campestris, hyphæ, basidia, and spores, Beauverie (1914a, p. 800).
    Puccinia malvacearum, hyphæ, Beauverie (1914b, p. 359).
    Pustularia vesiculosa, asci, Guilliermond (1913a, p. 618; 1913b, p. 646).
    Rhyzopus nigricans, young spores and filaments, Moreau (1915b, p. 143).
    Saccharomyces cerevisiæ and ludwigi, cells, Guilliermond (1913g, p. 1781).
    Sporodinia grandis, zygospores, Moreau (1914a, p. 347).
Bryophytes:
    Anthoceras husnoti, several parts, Scherrer (1913, pl. 20).
    Bryum ——, Sapehin (1915, pls. 25, 26).
    Funaria hygrometrica, leaves, Sapehin (1915, pls. 22, 23, 25, 26).
    Polytrichium 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).

Scolopendrium 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 (1912e, 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).

Curcurbita 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 foetidus, 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).

Nymphæa alba, tapetum, Meves (1904, p. 284).

Oenothera biennis, several parts, Sapehin (1915, pls. 11, 12).

Papaver rhœas, 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).

Tropæolum lobbeanum, 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).

Vanillia 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 electosome theory (p. 118).

SUPRAVITAL STAINING.

The most satisfactory dyes for this purpose are janus green B, janus blue, janus black I, and diethylsafranin (Cowdry, 1916a, 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, 1914b, 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 4μ, 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 (1916b, 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 picric 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-

---

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 pyroligneous 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 5μ.

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, embed, 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-ferricyanide 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, 1913a, 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 iodic-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 (1911a, p. 465), Maximow (1916a, 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 mito-chondria 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-ducts. 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 rela-tively 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's[1] 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's[2] observation that it is difficult to alter the distribu-tion of cytoplasmic materials in nerve-cells by centrifuging indicates the improb-ability of any considerable protoplasmic streaming.

Rubaschkin's (1910, p. 428) idea that filamentous mitochondria are char-acteristic of specialized cells and granular ones of embryonic, undifferentiated cells has been negatived 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 (1914b, 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 filament-ous 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 lipoid 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 lipoid 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 lipoid-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 algæ.

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. Beckwith'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.
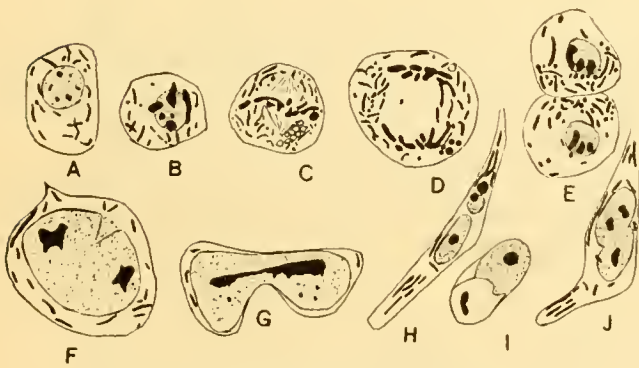
Fig. 1.—Dividing cells in chick embryo. A–E in mitosis and F–J in apparent amitosis.
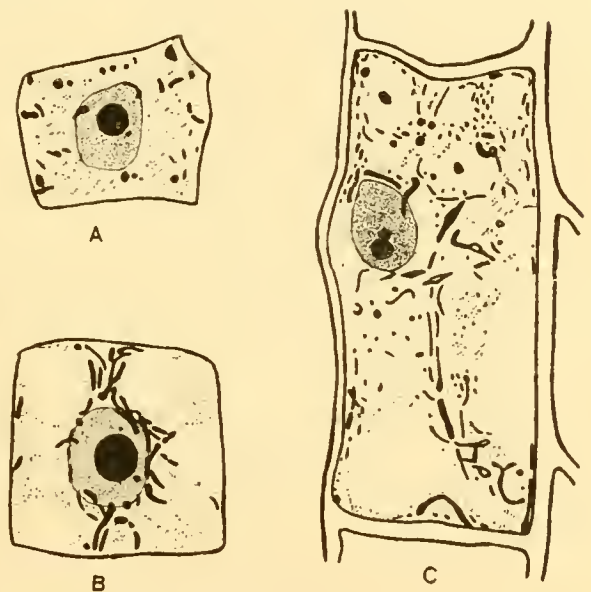
Fig. 2.—Meristem and young and old cortical cells of the pea, showing primary diffuse arrangement of mitochondria (A), secondary condensation about the nucleus (B), and final even distribution throughout the cytoplasm (C). (After N. H. Cowdry.)

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.
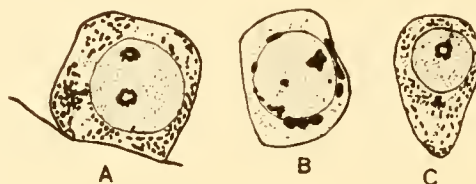
Fig. 3.—Spermatogonium, spermatocyte, and spermatid of mouse, showing (A) similar diffuse arrangement of mitochondria, (B) secondary circumnuclear condensation, (C) final even distribution in the cytoplasm. (After N. H. Cowdry, 1917.)

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-

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 amœboid 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.

----

[1] N. 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.)
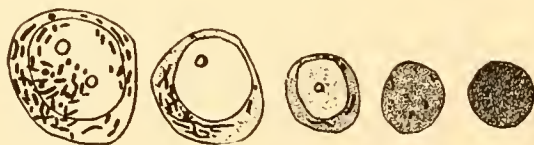
FIG. 5.—Erythroblast, megaloblast, normoblast, and erythrocytes from bone marrow of a rabbit stained supravitally with janus green, showing the parallelism between the disappearance of mitochondria and the appearance of hemoglobin in the form of a diffuse deposit. (After N. H. Cowdry, 1917.)

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 plasts are fully formed few if any mitochondria remain (Guilliermond, 1912a, 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 mitchondria 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
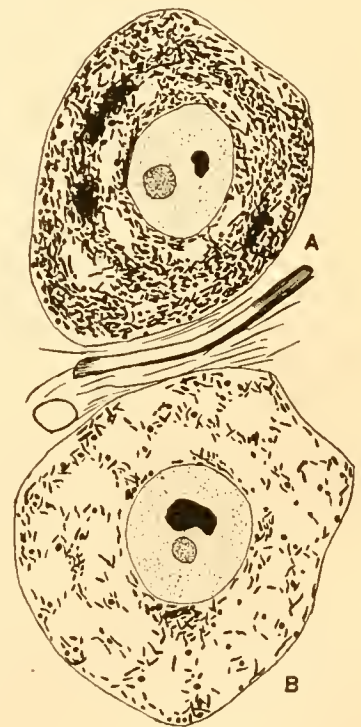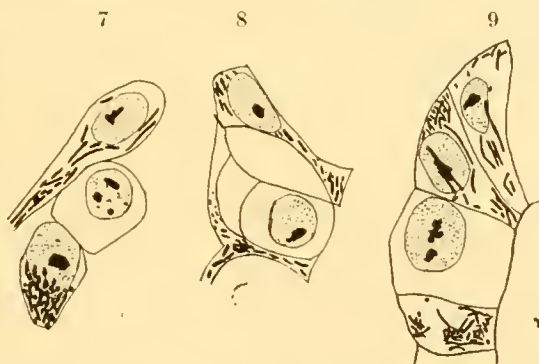

Fig. 6.—Spinal ganglion cells of the pigeon, showing (A) great increase in the amount of mitochondria as contrasted with the average amount (B).

show no evidence of fatty substances on histological examination may neverthe-less 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 (1908a, p. 17), and the cells of the glomeruli of embry-onic human kidneys (Policard, 1912c, p. 442; 12f, p. 12) have never been found to contain mitochondria.   Branca (1911, p. 559) has failed to find them in the in-ferior or germinative zone of hairs.   Other cases of the apparent absence of mito-chondria may be cited.

FIGS. 7, 8, and 9.—Neural tube, myotome, and Wolffian duct, showing cells apparently normal yet devoid of mitochondria.

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 mito-chondria 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

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 (1908d, p. 720), in the first place in the study of mammalian tissues, Fauré-Fremiet (1910a, p. 622), who worked on protozoa, and the botanist Löwschin (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 Löwschin 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 Polieard (1912d, 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 (Key[1]). 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 lipoid; 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

---

[1]Personal 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 diethylsafraninazodimethylanilin, 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.
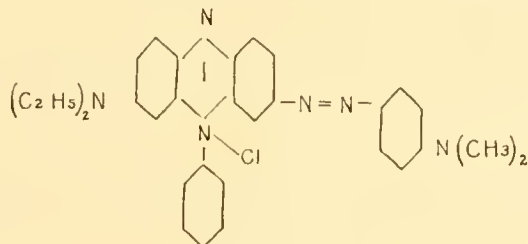
FIG. A.

Laguesse immediately recognized the importance of Michaelis's discovery, obtained some janus green from Grübler (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 leucobase.

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 restain the mitochondria with either diethylsafranin or janus green.

The reaction of mitochondria to janus green is essentially similar in other tissues.   I have elsewhere (1914b, 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, lipoid 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

$(C_2 H_5)_2 N -$ ... $- N = NOH \; + \; \bigcirc - N (CH_3)_2 \;\ggg\longrightarrow$
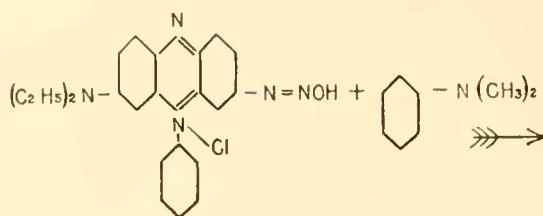
Fig. B.

Diazingrün (janus green):

$(C_2 H_5)_2 N$ ... $- N = N - \bigcirc N (CH_3)_2 \; + H_2O \;\ggg\longrightarrow$
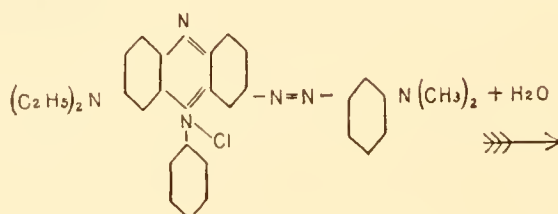
Fig. C.

Diethylsafraninazodimethylanilinchloride:

$(C_2 H_5) N -$ ... $- N = N - \bigcirc N (CH_3)_2$
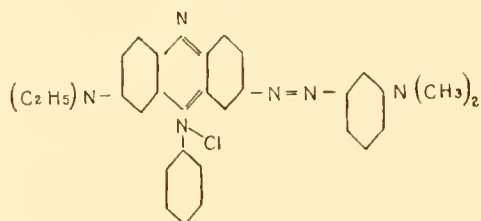
Fig. D.

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 formulæ, which makes three varieties of janus green in all:

(1) Janus green (Grübler), safraninazodimethylanilinchloride:

H_2N ... $N = N \bigcirc N (CH_3)_2$
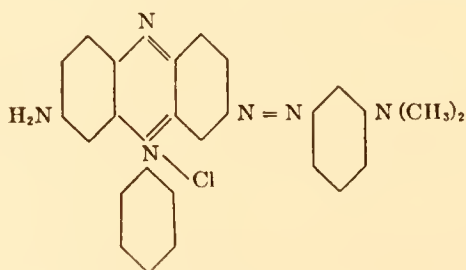
Fig. E.

(2) Janus green C (Farbwerke Hoechst Company), dimethylsafraninazodimethylanilinchloride:
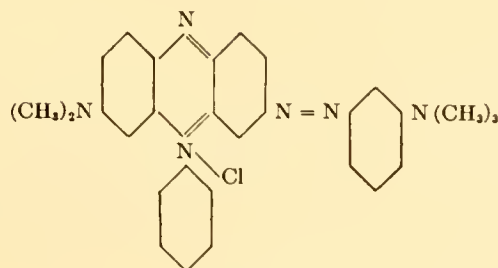
FIG. F.

(3) Janus green B (Farbwerke Hoechst Company), diethylsafraninazodimethylanilinchloride:
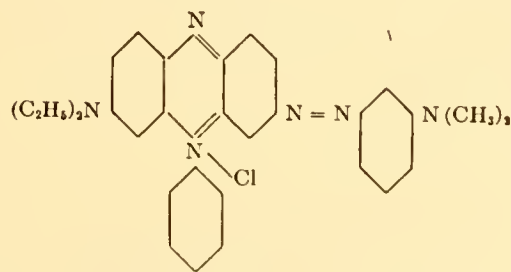
FIG. G.

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 Grübler, 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 Grübler, 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 clematin (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

---

[1] Dr. 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 diethylsafranin 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 diethylsafranin 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 strains.*

	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)	#	#	#	#	#	#	+	(?)	—	Gives a specific stain of mitochondria only.
Janus blue (Hoechst)	#	#	#	+	—					Acts in the same way but rather more slowly.
Janus black 1 (Hoechst)	#	#	#	(?)	—					Same as janus green B.
Diethylsafranin (Hoechst)	#	+	—							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.
Bismarck 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.
Pyrrolblau (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 mitochrondria, 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 (1911b, p. 709) on *Ascaris megalocephala*, confirmed by Held (1912, p. 247), Romeis (1913b, p. 166), and Fauré-Fremiet (1913, p. 585); those of Meves on *Parechinus miliaris* (1912, p. 102), *Phallusia mamillata* (1913, p. 225), *Filaria papillosa* (1915b, p. 58), and *Mytilus edulis* (1915c, 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 (1912a, 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 plasts 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 (1910a, 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, 1916b, 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 Beckwith (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. Beckwith 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 Beckwith'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, Beckwith 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 *Aurelia 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, 1912a, p. 289, and others). Following this line of reasoning, Hoven, (1910a, p. 478) and Meves (1910b, 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, Guilliermond (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, Guilliermond (1912a, p. 389).
Cerebrospinal fluid, Grynfeltt and Euzière (1912, p. 64).
Chloroleucites (= chloroplasts).
Chlorophyll, Guilliermond (1912a, p. 407).
Chloroplasts, Guilliermond (1912a, p. 412).
Chromoplasts, Guilliermond (1912a, p. 412).
Ciliary apparatus, Saguchi (1917, p. 266).
Collagenic fibrils, Meves (1910a, p. 164).
Composés phenolique, Guilliermond (1913f, p. 1002).
Connective-tissue cell granulations, Renaut and Dubreuil (1906b, p. 230).
Cuticular formations, Van der Stricht (1918, p. 36).
Cyanoplasts, Guilliermond (1913i, p. 1282).
Disque Q, Fauré-Fremiet, Mayer and Schaeffer (1910, p. 75).
Eberth's intracellular structures, Saguchi (1913, p. 239).
Elaïoplasts, Guilliermond (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, Perroncito (1910, p. 310).
Fucasin, Le Touze (1912, p. 33).
Glycogen, Arnold (1908, p. 365); Alexeieff (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, Guilliermond (1913d, p. 439).
Mucorine, Moreau (1915a, p. 171).
Mucus (boules picriphiles), 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 chrysomelid eggs, Hegner (1914, p. 419).
Prepucial-gland secretion, Altmann (1890, p. 99).
Prostate secretion, Akatsu (1903, p. 566).
Randreifen 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, Guilliermond (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, Guilliermond, (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, Guilliermond (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 eclectosome theory (1909a, 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, lipoid, 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 (1910a, 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 Chondrioconten hervorgehen. Die Chondrioconten 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 deteiligen 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 (chondriocontes) 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 chondriocontes 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 (1911a, 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 (1899a, p. 379) and Meves (1907a, 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 (1910a, p. 476), Prenant (1911a, p. 463), G. Arnold (1912a, p. 289), Schäfer (1912, p. 193), Luna (1913c, 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 (1913a, 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 (1916c, 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, 1916a, 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 $6\frac{1}{2}$ 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-

iliforme'' 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.	FIBRILS.
1. Granules, rods, filaments.	Long threads of finer diameter (Firket, p. 544).
2. Soluble in fluids containing an excess of acetic acid, e. g., Zenker's fluid.	Resistant to acetic acid in fixatives.
3. Destroyed by fixation in Bouin's fluid (Firket).	Well preserved by Bouin's fluid (Firket).
4. Acidophile in pancreas after fixation in non-mordanting fluids (Bensley, 1911, p. 362).	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.

<div align="center">NEUROFIBRILS.</div>

Many investigators have touched on the question whether mitochondria play a part in the differentiation of neurofibrils, but Hoven (1910a, 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 (1907a, p. 403), as well as by G. Arnold (1912a, 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, 1910a, p. 478; Meves, 1910b, 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, 1910a, 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 (1910a, 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, 1907a, p. 403; Duesberg, 1910, p. 613; Meves, 1910a, p. 162; Firket, 1911, p. 545; and Duesberg, 1912, p. 759).

The bearing of my own observations (1914d) 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 (Kite[1]). 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.

---

[1] Dr. 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 Herxheimer* 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 ciliæ, 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 (1912a, 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 (1912a, 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 "carotine" 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 algæ 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 plasts. It is necessary
to find out whether the chloroplasts in animals, some varieties of *Paramœcium*
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 transforma-
tion 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, con-
tain typical mitochondria. In October, also, Meves (1911a, 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, con-
cluding that the plastosomes (mitochondria) are the formers of the hemoglobin.

This was followed almost immediately by a second paper by Ciaccio (1913b,
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-corpuscles. There is either a deficiency in the formation of hemoglobin or an increase in its destruction. At any rate, the red blood-corpuscles 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 Grynfeltt 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 lipoid-like droplets in the cells. He demonstrated the mitochondria by mitochondrial methods which he does not specify and the lipoid by the method of Ciaccio. He assumes that the lipoid 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 Grynfeltt 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 (1912e, 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 (1913a, 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 (1913b, 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 (1909b, 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 "plastes." 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 (1912a, 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 (1912b, 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 lipoid 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 chondriocontes, 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 "plastes," 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, lipoid, 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 nucleoalbumin, 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 *Pyrrhocris 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 mito-
chondrial 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
lipoid 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 fre-
quently 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 (1910b, 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, "Olkügeln," 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 (1910b, p. 114) studied the mitochondria in the fully developed retinæ of a number of vertebrates, including man (1910c, 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 retinæ 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 iodic-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 Renaut, 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 (1906b, p. 230) call attention to the following observations: (1) that the périnéme (i. e., the mitochondrial apparatus) is rudimentary in young forms like the small rhagiocrine 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 clasmatocytes, 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 mito-
chondria play the part of "eclectosomes" (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 morpho-
logical 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 state-
ments 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 produc-
tion, 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, particu-
larly with reference to the lymphocytes; because if the lymphocytes do not trans-
form into the other connective-tissue cells, as they believe, there is nothing signifi-
cant 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 inves-
tigators 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 (chondrio-
contes) between the nucleus and distal cell border, a subsequent migration toward
the distal cell border and a transformation into rod-like corpuscles 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 "eclectosome" 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 rom 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 (1916a, 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 herbivors as in carnivors; 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 lipoid 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 (1916a, 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:

Adenoma of thyroid, Goetsch (1916, p. 132).
Anemic necrosis, Israel (1891, p. 310).
Asphyxiation in tissue culture, Champy (1914. p. 220).
Autolytic changes, Dannehl (1892, p. 485).
Beri-beri, Clark (1914, p. 92).
Carcinoma:
    Beckton (1909, p. 191).
    Porcelli-Titone (1914, p. 237).
    Favre and Regaud (1913, p. 688).
    Lubarsch (1897, p. 640) and others.
Cicatrization in tissue culture, Champy (1914. p. 368).
Cloudy swelling in kidney, Schilling (1894, p. 470).
Diabetes, Homans (1915, p. 16).
Diphtheria toxin, d'Agata (1913, p. 443); Dibbelt (1914, p. 119).
Diuresis, Policard (1910, p. 272).
Edema of prepuce, Regaud and Favre (1912. p. 330).
Epithelioma, G. Arnold (1012a, p. 283); Favre and Regaud (1913, p. 688).
Fatty degeneration, kidney, Ophüls (1907. p. 136).
Fatty infiltration, Altmann (1889, p. 94).
Feltphanrosis, nerve-cells, Biondi (1915, p. 224).
Galls (nematode) in plants, Nemec (1910, p. 166).
Hemoglobinuria, kidney, Barratt (1913, p. 566).
Hypertrophic tonsils and adenoids, Alagna (1911, p. 27).
Inanition, Russo (1910, p. 173).

Inflammation, connective tissue, Dubreuil (1913, p. 138).
Irritation of cortex, Collin (1914, p. 592).
Liver extirpation, effect on mitochondria in kidney, Policard (1910, p. 245).
Myelogenous leukemia, Klein (1910, p. 407).
Pernicious anemia, spleen, Shipley (1915, p. 75).
Phosphorus poisoning, Lubarsch (1897, p. 639); Scott (1916, p. 237); Mayer, Rathery and Schaeffer (1914, p. 607), etc.
Pigmentation, Barratt (1913, p. 566).
Poisons, various, Mayer, Rathery and Schaeffer (1914, p. 607).
Polypus of nose, Benda (1899a, p. 380).
Prostatitis, Dominici (1913, p. 295).
Radium, Beekton and Russ (1911, p. 99).
Regeneration, Romeis (1913c, p. 1); Torraca (1914a, p. 539; 1914b, p. 459).
Rodent ulcer, Regaud and Favre (1912, p. 329).
Sarcoma treated with X-ray, Regaud (in discussion of Laguesse) (1912b, p. 111).
Scharlach R, skin proliferation, Barratt (1913, p. 566).
Suprarenal gland after ovariectomy, Mira (1912, p. 43).
Toxins, Mayer, Rathery and Schaeffer (1914, p. 609).
Tubercle giant cell, Champy (1911, p. 154).
Uranium nephritis, Oliver (1916, p. 306).
X-ray, Beckton and Russ (1911, p. 99).

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 phagocytosis 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 (1913g, 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 (1912d, 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 anyth'ng 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 dyspnœa 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 (1910a, 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 (1912a, 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, chefly 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 Beckton 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 de-differentiation 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 chromatins 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, lipoid, 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, lipoid, 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 (1908*a*, 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 *Trochelminthcs*, 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.	Temper-ature.	Calories.	Determi-nation.	Authority.
		°C.			
Tenebrio larva..........	0.1	18	1.45	O_2	Thunberg.
Formica.......	0.01	20	2.5	O_2	Slowtzoff.
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 anærobic and which normally live in the absence of oxygen. *Ascaris* is a good example. According to Mathews (1905, p. 333):

"The only difference between anærobic and ærobic respiration is that the anærobic 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. Ærobic 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 (1913a, 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 Grynfeltt (1912b, 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 cœruleus 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.
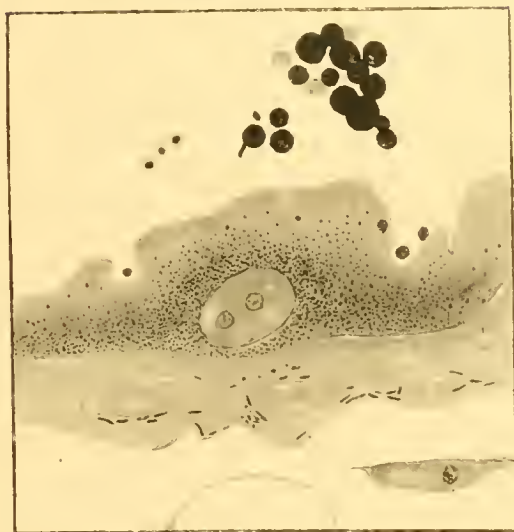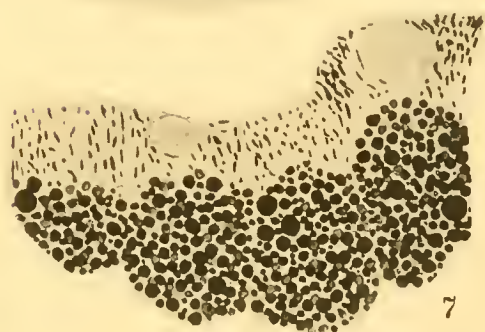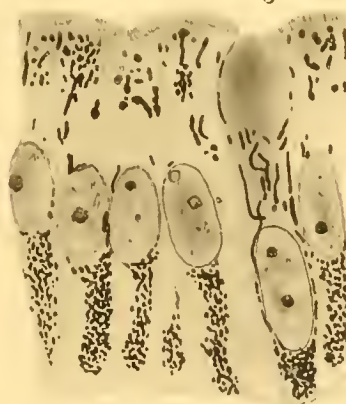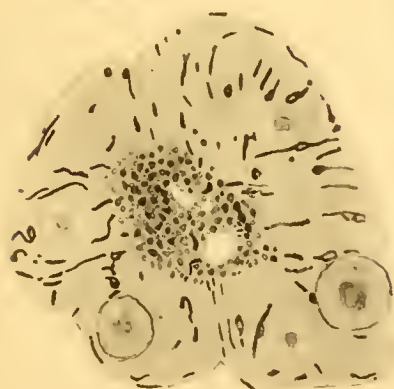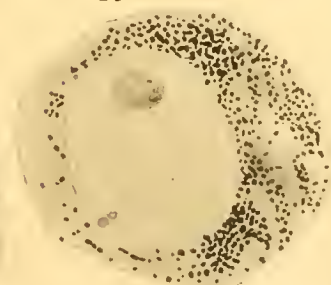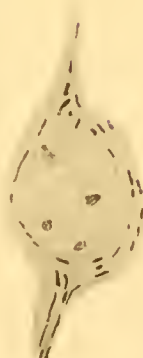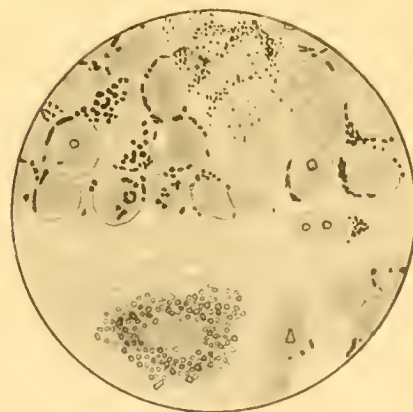
PLATE 1

1
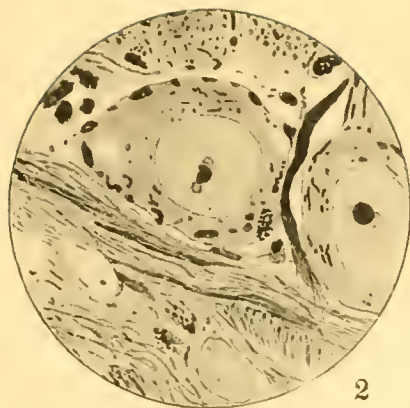
2
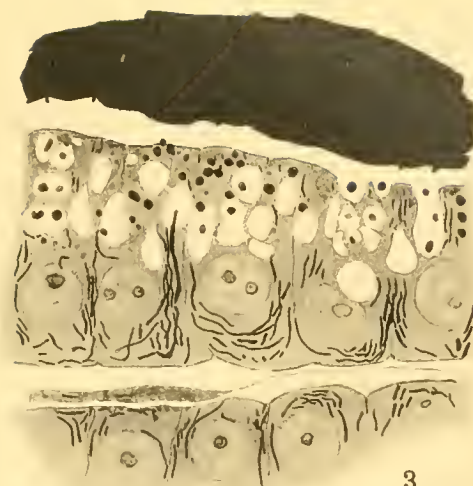
3

4

5

7

11

10

8

9

6

12

13

BIBLIOGRAPHY.[1]

AIMÉ, PAUL, 1911. Bandes intercalaires et bandes de contraction. Bibl. Anat., t. 21, pp. 263–272 (see text, p. 56).

AKATSU, 1903. Mikroskopische Untersuchung der Sekretionsvorgänge in den Samenblasen. Pflüger's Archiv, Bd. 96, pp. 555–571 (pp. 51, 102, 119).

ALAGNA, A., 1911. Sulla presenza di formazioni mitocondriali negli elementi constitutivi delle tonsille palatine normali ipertrofiche e delle vegetazioni adenoidi. Arch. f. Zellforsch., Bd. 7, pp. 27–40 (pp. 51, 52, 135).

ALEXEIEFF, A., 1916a. Mitochondries chez quelques protistes. Mitochondries glycoplastes et adipoplastes. Caractères généraux des mitochondries. C. rend. Soc. Biol., t. 79, pp. 1076–1079 (p. 102).

———— 1916b. Mitochondries chez quelques protistes. Mitochondries glycoplastes. C. rend. Soc. Biol., t. 79, pp. 1072–1075 (p. 53).

———— 1917a. Sur les mitochondries a fonction glycoplastique. C. rend. Soc. Biol., t. 80, pp. 510–512 (p. 102).

———— 1917b. Nature mitochondriale du corps parabasal des Flagellés. C. rend. Soc. Biol., t. 80, pp. 499–502 (pp. 46, 53).

———— 1917c. Mitochondries et corps parabasal chez les Flagellés. C. rend. Soc. Biol., t. 80, pp. 358–360.

ALTMANN, R., 1889. Ueber die Fettumsetzungen im Organismus. Arch. f. Anat. u. Phys., Anat. Abt., Suppl. Bd., pp. 86–104 (pp. 102, 124, 135).

———— 1890. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Leipzig, Veit Co., 145 pp. (pp. 42, 44, 51, 52, 59, 101, 102, 135, 142).

———— 1894. Die Elementarorganismen und ihre Beziehungen zu den Zellen. 2. Aufl. Leipzig, Veit Co., 160 pp. (pp. 46, 51, 97).

ARNDT, A., 1914. Ueber generative Vorgänge bei Amœba chondrophora, n. sp. Arch. f. Protistenkunde, Bd. 34, pp. 39–59 (pp. 44, 49, 53).

ARNOLD, GEORGE, 1912a. On the condition of the epidermal fibrils in epithelioma. Quart. Jour. Micr. Sci., vol. 57, pp. 283–299 (pp. 101, 107, 110, 135, 140).

———— 1912b. The rôle of the chondriosomes in the cells of the guinea-pig's pancreas. Arch. f. Zellforsch., Bd. 8, pp. 252–271 (pp. 48, 121).

ARNOLD, J., 1907. Plasmosomen, Granula, Mitochondrien, Chondriomiten und Netzfiguren. Anat. Anz., Bd. 31, pp. 640–648 (p. 46).

———— 1908. Supravitale Färbung Mitochondrien ähnlicher Granula in den Knorpelzellen nebst Bemerkungen über die Morphologie des Knorpelglykogens. Anat. Anz. Bd. 32, pp. 361–366 (pp. 102, 127).

———— 1913. Das Plasma der somatischen Zellen im Lichte der Plasmosomen-Granulalehre und der Mitochondrienforschung. Anat. Anz., Bd. 43, pp. 433–462 (p. 46).

ASVADOUROVA, NINA, 1913. Recherches sur la formation de quelques cellules pigmentaires et des pigments. Arch. d'Anat. micr., t. 15, pp. 153–314 (pp. 45, 49, 84, 102).

AUNAP, E., 1913. Ueber die Chondriosomen der Gonocyten bei Knochenfischen. Anat. Anz., Bd. 44, pp. 449–459 (p. 56).

AZZI, AZZO, 1914. Ueber das Verhalten der Chondriosomen bei der fettigen Entartung. Centralbl f. allg. Path. u. path. Anat., Bd. 25, pp. 7–13 (p. 124).

BABCOCK, S. M., 1912. Metabolic water; its production and rôle in vital phenomena. Univ. of Wisconsin Agric. Exper. Stat. Res. Bull. No. 22, pp. 87–187 (p. 145).

VON BAEHR, W. B., 1909. Die Oogenese bei einigen viviparen Aphididen und die Spermatogenese von Aphis saliceti, mit besonderer Berücksichtigung der Chromatinverhältnisse. Arch. f. Zellforsch, Bd. 3, pp. 269–334 (p. 55).

BAITSELL, G. A., 1916. The origin and structure of a fibrous tissue formed in wound healing. Jour. Exper. Med., vol. 23, pp. 739–756 (p. 105).

BALLOWITZ, E., 1907. Zur Kenntnis der Spermien der Cetaceen. Arch. f. mikr. Anat., Bd. 70, pp. 227–237 (pp. 44, 56).

BARRATT, J. O., 1913. Changes in chondriosomes occurring in pathological conditions. Quart. Jour. Micr. Sc., vol. 58, pp. 553–566 (p. 136).

BAUMGARTNER, W. J., 1902. Spermatid transformations in Gryllus assimilis, with special reference to the nebenkern. Kansas Univ. Sci. Bull., vol. 1, pp. 47–74 (p. 55).

BAYLISS, W. M., 1915. Principles of General Physiology. London, Longmans, Green & Co., 850 pp. (pp. 134, 145–147).

BEAUVERIE, J., 1914a. Sur le chondriome des Basidiomycètes. C. rend. Acad. Sci., t. 158, pp. 798–800 (pp. 52, 56).

———— 1914b. Sur le chondriome d'une Urédinée: le Puccinia malvacearum. (Reun. biol. Nancy.) C. rend. Soc. Biol. t. 76, pp. 359–361 (pp. 52, 56).

BECKTON, H., 1909. On granules in cells of normal tissues and new growths. Arch. Middlesex Hosp., vol. 15, pp. 182–191 (pp. 135, 139).

———— and RUSS, S., 1911. The effect of radium emanation on Altmann's granules. Arch. Middlesex Hosp., vol. 23, pp. 59–105 (pp. 135, 140).

BECKWITH, C. J., 1914. The genesis of plasma-structures in the egg of Hydractinia echinata. Jour. Morph., vol. 25, pp. 189–252 (pp. 54, 74, 97, 98).

BENDA, C., 1897. Neue Mitteilungen über die Histogenese der Säugetierspermatozoen. Verh. d. physiol. Ges., pp. 406–414 (pp. 94, 102).

———— 1898. Ueber die Spermatogenese der Vertebraten und höheren Invertebraten. 2. Die Histogenese der Spermien. Verh. d. physiol. Ges., pp. 393–398 (pp. 45, 49).

---

[1] The bibliography is not complete, for only the papers cited in the text are given. Duesberg (1912) has given a complete list of references to 1911.

BENDA, C., 1899a. Weitere Mitteilungen über die Mitochondria. Verh. d. Physiol. Ges., pp. 376–383 (pp. 42, 45, 47, 49, 52, 55, 102, 106, 135).

—— 1899b. Weitere Beobachtungen über die Mitochondria und ihr Verhältnis zu Secretgranulationen, nebst kritischen Bemerkungen. Verh. d. physiol. Ges. (pp. 51, 54).

—— 1901. Die Mitochondriafärbung und andere Methoden zur Untersuchung der Zellsubstanzen. Verh. d. anat. Ges., pp. 155–174 (p. 61).

—— 1902. Die Mitochondria. Ergebn. d. Anat. u. Entw. (Anat. Hefte, 2. Abt.), Bd. 12, pp. 743–781 (p. 75).

—— 1903. Die Mitochondrien des Nierenepithels. Verh. d. anat. Ges., pp. 123–129 (pp. 122, 130).

—— 1914. Die Bedeutung der Zellleibstruktur für die Pathologie. Verh. d. deutsch. path. Ges., pp. 5–42 (pp. 48, 50, 130).

BENSLEY, R. R., 1910a. On the so-called Altmann's granules in normal and pathological tissues. Trans. Chicago Path. Soc., vol. 8, pp. 78–83 (p. 139).

—— 1910b. On the nature of the canalicular apparatus of animal cells. Biol. Bull., vol. 19, pp. 179–194 (p. 44).

—— 1911. Studies on the pancreas of the guinea-pig. Amer. Jour. Anat., vol. 12, pp. 297–388 (pp. 44, 51, 59, 60, 62, 87, 109).

—— 1916. The normal mode of secretion in the thyroid gland. Amer. Jour. Anat., vol. 19, pp. 37–54 (pp. 52, 63, 73, 148).

BINFORD, RAYMOND, 1913. The germ-cells and the process of fertilization in the crab, Menippe mercenaria. Jour. Morph., vol. 24, pp. 147–200 (p. 54).

BIONDI, G., 1915. Ueber die Fettphanerosis in der Nervenzelle. Virchow's Archiv, Bd. 220, pp. 222–233 (pp. 135, 137).

BLÜNTSCHLI, H., 1904. Beobachtungen am Ovarialei der Monascidie Cynthia microcosmus. Morph. Jahrb., Bd. 32, pp. 391–449 (p. 55).

BOBEAU, G., 1911. Mitochondries et lipoides dans les glandules parathyroïdes du cheval. C. rend. Ass. Anat., pp. 183–187 (pp. 52, 102, 117).

BONNEVIE, K., 1904. Zur Kenntnis der Spermiogenese bei den Gastropoden (Enteroxenes östergeni). Biol. Centralbl., Bd. 24, pp. 267–274 (p. 55).

—— 1907. Untersuchungen über Keimzellen. II. Physiologische Polyspermie bei Bryozoen. Jena Zeitschr. f. Naturw., Bd. 42, pp. 567–598 (p. 54).

BOVERI, TH., 1888. Zellen-Studien. Jena Zeitschr. f. Naturw., Bd. 22, pp. 686–882 (p. 44).

BRANCA, A., 1911. Sur la structure du poil. Jour. de l'Anat et de la Phys., t. 47, pp. 545–600 (pp. 51, 81).

BROMAN, J., 1907. Ueber Bau und Entwickelung der Spermien von Rana fusca. Arch. f. mikr. Anat., Bd. 70, pp. 330–360 (p. 56).

BROWNE, ETHEL N., 1913. A study of the male germ-cells in Notonecta. Jour. Exper. Zool., vol. 14, pp. 61–121 (p. 55).

BRÜCK, A., 1914. Die Muskulatur von Anodonta cellensis Schröt. Zeit. f. wiss. Zool., Bd. 110, pp. 481–619 (pp. 55, 107).

VON BRUNN, A., 1884. Beiträge zur Kenntnis der Samenkörper und ihrer Entwickelung bei den Säugetieren und Vögeln. Arch. f. mikr. Anat., Bd. 23, pp. 108–132 (pp. 45, 51).

BUCHNER, P., 1909. Das accessorische Chromosom in Spermatogenese und Ovogenese der Orthopteren. Arch. f. Zelforsch., Bd. 3, pp. 335–430 (p. 55).

BULLARD, H. H., 1916. On the occurrence and physiological significance of fat, etc. Amer. Jour. Anat., vol. 19, pp. 1–35 (p. 83).

BURROWS, M. T., 1917. Some factors regulating growth. Anat. Rec., vol. 11, pp. 335–339 (p. 79).

BUSACCA, A., 1913. L'apparato mitochondriale nelle cellule nervose adulte. Arch. f. Zellforsch., Bd. 11, pp. 327–339 (p. 56).

—— 1915. Sulle modificazione dei plastomi nelle cellule del epitelio pigmentato della retina sotto l'azione dell' luce e dell' oscurita (Tabola 11). Ricerche fatte nel Lab. di Anat. normale della Univ. di Roma., vol. 18, pp. 217–237 (p. 132).

CAJAL, S. R., 1908. Les conduits de Golgi-Holmgren du protoplasma nerveux et le réseau péricellulaire de la membrane. Trab. Lab. Invest. biol. Univ. Madrid, t. 6, pp. 123–136 (p. 45).

—— 1915. Algunas variaciones fisiologicas y patologicas del aparato reticular de Golgi. Trab. Lab. Invest. biol. Univ. Madrid, t. 12, 101 (pp. 63, 125).

CASTEEL, D. B., 1917. Cytoplasmic inclusions in male germ-cells, etc. Jour. Morph., vol. 28, pp. 643–686 (p. 55).

CERMAK, N., 1902. Die Mitochondrien des Forelleneies. Anat. Anz., Bd. 20, pp. 158–160 (p. 56).

CESA-BIANCHI, D., 1909. Experimentelle Untersuchungen über die Nierenzelle. Frankfurt Zeitschr. f. Path., Bd. 3, p. 461 (p. 137).

—— 1910. Contributo alla conoscenza della anatomia e della fisio-patologia renale. Internat. Monatsschr. f. Anat. u. Phys., Bd. 27, pp. 89–186 (p. 137).

CESARIS-DEMEL, H., 1907. Studien über die roten Blutkörperchen mit den Methoden der Färbung in frischem Zustande. Folia hæm., Bd. 4, Suppl., pp. 1–32 (p. 46).

CHAMBERS, R., 1915. Microdissection studies on the germ cell. Science, n. s., vol. 41, pp. 290–293 (pp. 43, 97, 98).

CHAMPY, C., 1911. Recherches sur l'absorption intestinale et le rôle des mitochondries dans l'absorption et la sécrétion. Arch. d'Anat. micr., t. 13, pp. 55–170 (pp. 121, 133, 135).

—— 1912. Sur les phénomènes cytologiques qui s'observent dans les tissus cultivés en dehors de l' organisme (tissus épithéliaux et glandulaires). C. rend. Soc. Biol., t. 72, p. 987 (pp. 43, 52, 147).

—— 1913a. Recherches sur la spermatogenèse des Batraciens et les elements accessoires du testicule. Arch. de Zool. exp. et. gén., t. 52, pp. 13–304 (pp. 45, 49, 56).

—— 1913b. La dédifférenciation des tissus cultivés en dehors de l'organisme. Bibl. Anat., t. 23, pp. 184–205 (pp. 43, 52).

—— 1914. IV. Le rein. Arch. de Zool. exp. et gén., t. 54, pp. 307–386 (pp. 44, 52, 135).

—— 1915. V. La glande thyroïde. Arch. de Zool. exp. et gén., t. 55, pp. 61–79 (p. 52).

CHAVES, P. R., 1912. Notes sur l'ergastoplasme. Bull. Soc. Portugaise Sci. Nat., t. 6, pp. 29–33 (p. 56).

—— 1915. Sobre a cellula serosa pancreatica. Arch. de Anat. e de Anthrop., vol. 4, pp. 1–131 (pp. 56, 121).

CHILD, C. M., 1915. Senescence and rejuvenescence. Univ. of Chicago Press. 481 pp. (p. 74).

CIACCIO, C., 1911. Sui mitochondrî degli elementi linfoidi e mieloidi. Pathologica, vol. 3, pp. 13–17 (pp. 102, 115).

——— 1913a. Zur Physiopathologie der Zelle. Centralbl. f. allg. Path. u. path. Anat., Bd. 24, pp. 721–727 (pp. 46, 115).

——— 1913b. Les plastosomes des éléments de la série hémoglobinique. Folia hæm., pp. 391–393.

CIACCIO and SCAGLIONE, 1913. Beitrag zur cellulären Physiopathologie der Plexus chorioidei. Ziegler's Beiträge z. path. Anat., Bd. 55, pp. 131–167 (p. 118).

CLARK, ELBERT, 1914. Regeneration of medullated nerves in the absence of embryonic nerve fibers following experimental non-traumatic degeneration. Jour. Comp. Neur., vol. 24, pp. 61–111 (pp. 135, 137).

COGHILL, G. E., 1915. Preliminary studies in intracellular digestion and assimilation in amphibian embryos. Science, n. s., vol. 42, pp. 347–350 (pp. 102, 124, 133).

COLLIN, R., 1913a. Les mitochondries de la cellule névroglique à expansions longues et les granulations lipoïdes de la substance grise des centres nerveux chez l'homme. C. rend. Assoc. Anat., pp. 178–186 (p. 52).

——— 1913b. Les mitochondries du cylindraxe, des dendrites et du corps des cellules ganglionnaires de la rétine. C. rend. Soc. Biol., t. 74, pp. 1358–1360 (p. 52).

——— 1914. Sur les mitochondries extraneuronales dans l'écorce cérébrale irritée. C. rend. Soc. Biol., t. 67, pp. 591–593 (p. 135).

COMOLLI, A., 1913. Ricerche istologiche sull' interrenale dei Teleostei. Arch. Ital. di Anat. e di Embriol., t. 11, pp. 377–408 (p. 56).

CONKLIN, E. G., 1905. Organ-forming substances in the eggs of ascidians. Biol. Bull., vol. 8, pp. 205–230 (pp. 44–46, 99).

——— 1915. Heredity and environment. Princeton University Press, pp. 533 (pp. 45, 96).

——— 1917. Effects of centrifugal force on the structure and development of the eggs of Crepidula. Jour. Exper. Zool., vol. 22, pp. 311–420 (pp. 45, 46).

CORNER, G. W., 1914. The corpus luteum of pregnancy, as it is in swine. Carnegie Inst. of Wash. Contributions to Embryology, No. 5, pp. 71–94 (p. 51).

CORTI, A., 1913. Studi sulla minuta struttura della mucosa intestinale di Vertebrati, etc. Arch. Ital. di Anat. e di Embriol., t. 11, pp. 1–189 (pp. 55, 56).

COWDRY, E. V., 1911. A preliminary report on the structure of nerve-cells. Jour. Nerv. and Ment. Dis., vol. 38, p. 753 (p. 46).

——— 1912. Mitochondria and other cytoplasmic constituents of the spinal ganglion cells of the pigeon. Anat. Rec., vol. 6, pp. 33–38 (p. 56).

——— 1913a. The relations of mitochondria and other cytoplasmic constituents in spinal ganglion cells of the pigeon. Internat. Monatschr. f. Anat. u. Phys., Bd. 29, pp. 473–504 (pp. 46, 63).

——— 1913b. Demonstration spéciales No. 6. Les mitochondries dans les cellules des ganglions spinaux traités par la méthode de Bensley. Bibl. Anat., t. 23, p. 311 (p. 46).

COWDRY, E. V., 1914a. The comparative distribution of mitochondria in spinal ganglion cells of vertebrates. Amer. Jour. Anat., vol. 17, pp. 1–29 (pp. 54, 55, 56, 102, 124).

——— 1914b. The vital staining of mitochondria, etc. Internat. Monatschr. f. Anat. u. Phys., Bd. 31, pp. 267–286 (pp. 52, 59, 88).

——— 1914c. The relations of mitochondria in cells multiplying by mitotic and amitotic division. Anat. Rec., vol. 8, pp. 102–103 (p. 79).

——— 1914d. The development of the cytoplasmic constituents of the nerve-cells of the chick. I. Mitochondria and neurofibrils. Amer. Jour. Anat., vol. 15, pp. 389–429 (pp. 110, 111).

——— 1916a. The general functional significance of mitochondria. Amer. Jour. Anat., vol. 19, pp. 423–446 (pp. 47, 59, 62, 108, 134).

——— 1916b. The structure of chromophile cells of the nervous system. Carnegie Inst. of Wash. Contributions to Embryology, No. 11, pp. 27–43 (pp. 60, 98, 131).

COWDRY, N. H., 1917. A comparison of mitochondria in plant and animal cells. Biol. Bull., vol. 33, pp. 196–228 (pp. 64, 69, 72, 77, 83, 86, 132).

D'AGATA, G., 1913. Sulla genesi del grasso e sulle modificazioni dell' apparato mitocondriale nell' intossicazione difterica. Internat. Monatschr. f. Anat. u. Phys., Bd. 29, pp. 443–459 (p. 135).

DA COSTA, C., 1913. Recherches sur l'histophysiologie des glandes surrénales. Arch. de Biol., t. 28, pp. 111–191 (pp. 52, 56).

——— 1916. Sur le développement des capsules surrénales du chat. Bull. Soc. Portugaise des Sci. Nat., t. 7, pp. 161–171.

D'ATHIAS, 1912. L'appareil mitochondrial des cellules interstitielles de l'ovaire du Murin. C. rend. Soc. Biol., t. 73, pp. 448–449.

——— 1915. Cristalloides dans l'œuf de Cercopithecus callitrichus et de Cercopithecus sabaeus. Bull. Soc. Portugaise des Sci. Nat., t. 7, pp. 67–76 (pp. 56, 116).

DANNEHL, P., 1892. Ueber die cadaverösen Veränderungen der Altmannschen Granula. Virchow's Arch., Bd. 128, pp. 485–496 (p. 135).

DE BONIS, V., 1907. Ueber die Sekretionserscheinungen in den Drüsenzellen der Prostata. Arch. f. Anat. u. Phys., Anat. Abt., pp. 1–16 (p. 120).

DIBBELT, 1914. Genese der Epithelveränderungen in der Niere bei experimenteller Diphtherie, ein Beitrag zur Pathologie der Zelle. Verh. d. deutsch. path. Ges., pp. 114–118.

DINGLER, M., 1910. Ueber die Spermatogenese des Dicrocodium lanceatum Stil. et Hass (Distomum lanceolatum). Arch. f. Zellforsch, Bd. 4, pp. 672–712 (p. 54).

DOMINICI, 1913. Sulle formazioni mitocondriali e sui granuli di secrezione nella prostata del cane e prostata umana ipertrofica. Folia urol., Bd. 7, pp. 295–303 (pp. 51, 119, 135, 137).

DUBREUIL, G., 1909. 1. Origine, destinée et appareil mitochondrial des Plasmazellen du grand épiploon du Lapin. C. rend. Soc. Biol., t. 67, pp. 80–82 (p. 52).

——— 1910a. Mitochondries des ostéoclastes et des cellules de Bizzozero. C. rend. Soc. Biol., t. 69, pp. 71–73 (p. 52).

——— 1910b. L'appareil mitochondrial (périnème, mitochondries, chondriochontes), dans la lignée cellulaire allant du lymphocyte à la cellule osseuse. C. rend. Soc. Biol., t. 68, pp. 1100–1102 (p. 52).

DUBREUIL, G. 1911a. Le chondriome des globules blancs mononucléées et des cellules connectives, cartilagineuses, et osseuses chez les mammifères. C. rend. Assoc. Anat., pp. 130–145 (p. 52).

———— 1911b. Transformation directe des mitochondries et des chondriocontes en graisse dans les cellules adipeuses. C. rend. Soc. Biol., t. 70, pp. 264–278 (p. 124).

———— 1912. La mitochondrie forme la plus apte à la multiplication des éléments du chondriome. C. rend. Assoc. Anat., pp. 127–133 (p. 48).

———— 1913. Le chondriome et le dispositif de l' activité sécrétoire, etc. Arch. d'Anat. micr., t. 11, pp. 53–151 (pp. 52, 62, 67, 127, 135).

DUBREUIL and FAVRE, 1914a. Nature et signification des corps de Russell. C. rend. Soc. Biol., t. 77, pp. 372–374 (p. 102).

———— 1914b. Chondriome des Plasmazellen. C. rend. Soc. Biol., t. 77, pp. 24–26 (p. 52).

DUESBERG, J., 1907. Der Mitochondrial-Apparat in den Zellen der Wirbelthiere und Wirbellosen. Arch. f. mikr. Anat., Bd. 17, pp. 284–296 (pp. 46, 50).

———— 1908. Sur l'existence de mitochondries dans l'oeuf et l'embryon d'Apis mellifica. Anat. Anz., Bd. 32, pp. 261–265 (p. 46).

———— 1909. Ueber Chondriosomen und ihre Verwendung zu Myofibrillen beim Hühnerembryo. Verh. d. anat. Ges., Bd. 34, pp. 123–126 (p. 106).

———— 1910. Les chondriosomes des cellules embryonnaires du poulet, et leur rôle dans la genèse des myofibrilles avec quelques observations sur le développement des fibres musculaires striées. Arch. f. Zellforsch., Bd. 4, pp. 602–671 (pp. 45, 46, 106, 110).

———— 1912. Plastosomen, "Apparato reticolare interno," und Chromidialapparat. Ergebn. d. Anat. u. Entw. Gesch. (Anat. Hefte. 2. Abt.), Bd. 20, pp. 567–916 (pp. 46, 48, 53, 97, 106, 109, 110, 126).

———— 1913. Plastosomes et "organ-forming substances" dans l'oeuf des ascidiens. Bull. de la classe des sci. Acad. roy. Belgique., No. 5, pp. 463–474 (p. 55).

———— 1915. Recherches cytologiques sur la fécondation des ascidiens et sur leur développement. Carnegie Inst. of Wash. Contributions to Embryology, No. 8, pp. 35–70 (pp. 48, 95, 98–100, 107).

———— 1917. Chondriosomes in the cells of fish embryos. Amer. Jour. Anat., vol. 21, pp. 465–496 (pp. 48, 49, 55, 60, 79).

DUESBERG, J., and H. HOVEN, 1910. Observations sur la structure du protoplasme des cellules végétales. Anat. Anz., Bd. 36, pp. 93–100 (p. 57).

DULZETTO, F., 1916. Contributo alla conoscenza della struttura della granulosa e dell' origine dei materiali deutoplasmici nell'oocite degli Uccelli (Fringilla cannabina L.). Arch. ital. di Anat. e di Embriol., vol. 15, pp. 193–217 (p. 56).

EKLÖF, H., 1914. Chondriosomenstudien an den Epithel- und Drüsenzellen des Magen-Darmkanals und den Oesophagus- Drüsenzellen bei Säugetieren. Anat. Hefte, 1. Abt., Bd. 51, pp. 1–227 (p. 51).

ENDERLEN, 1908. Beitrag zur Nierenchirurgie. Deutsche Zeitschr. f. Chir., Bd. 41, p. 208 (p. 137).

ERNST, P., 1914. Die Bedeutung der Zellleibstruktur für die Pathologie. Verh. d. deutsch. path. Ges., pp. 43–103 (p. 137).

FAHR, 1914. Zur Frage der sogenannten hyalintropfigen Zelldegeneration. Verh. d. deutsch. path. Ges., pp. 119–122 (p. 122).

FAURÉ-FREMIET, E., 1907. Mitochondries et sphéroplastes chez les Infusoires ciliés. C. rend. Soc. Biol., t. 62, pp. 523–525 (p. 46).

———— 1908. Évolution de l'appareil mitochondrial dans l'oeuf de Julus terrestris. C. rend. Soc. Biol., t. 64, pp. 1057–1058 (p. 55).

———— 1909. Sur les réactions de quelques mitochondries. C. rend. Acad. Sc., t. 149, pp. 163–166.

———— 1910a. Étude sur les mitochondries des Protozoaires et des cellules sexuelles. Arch. d'Anat. micr., t. 11, pp. 457–648 (p. 53–55, 83, 98, 123).

———— 1910b. Mitochondries et liposomes. C. rend. Soc. Biol., t. 68, pp. 537–539 (p. 102).

———— 1910c. Les glandes labiales des Hydrocorisis. C. rend. Assoc. Anat., pp. 1–4 (p. 51).

———— 1911. Le rôle des mitochondries dans l'élimination du fer chez les rhizopodes arénacés. C. rend. Soc. Biol., t. 70, pp. 119–136 (p. 102).

———— 1912a. Études cytologiques sur quelques Infusoires des marais salant du Croisic. Arch. d'Anat. micr., t. 13, pp. 402–480 (pp. 46, 54).

———— 1912b. Sur la constitution des mitochondries des gonocytes de l'Ascaris megalocephala. C. rend. Soc. Biol., t. 72, pp. 346–347 (pp. 54, 84).

———— 1913. Le cycle germinatif chez l'Ascaris megalocephala. Arch. d'Anat. micr., t. 15, pp. 435–757 (pp. 45, 83, 95, 99).

FAURÉ-FREMIET, E., ANDRÉ MEYER, et GEORGES SCHAEFFER, 1910. Sur la microchimie des corps gras. Application à l'étude des mitochondries. Arch. d'Anat. micr., t. 12, pp. 19–102 (p. 102).

FAVRE and DUBREUIL, 1914. Grains de ségrégation des Plasmazellen. C. rend. Soc. Biol., t. 77, pp. 89–91 (p. 128).

FAVRE, M., and CL. REGAUD, 1910. Sur la nature des fibres d'Herxheimer ou filaments basaux de l'épiderme. Lyon méd., No. 22, pp. 1132–1138 (pp. 45, 50, 51, 102, 107, 113).

———— 1913. Sur les formations mitochondriales dans les cellules néoplastiques des épithéliomes de la peau et des muqueuses dermopapillaires. C. rend. Soc. Biol., t. 74, pp. 688–692 (pp. 136, 140).

FIESSINGER and CAEN, 1910. Les altérations du chondriome chez les mammifères. C. rend. Soc. Biol., t. 68, pp. 454–455 (p. 127).

FIRKET, J., 1911. Recherches sur la genèse des fibrilles épidermiques chez le poulet. Anat. Anz., Bd. 38, pp. 537–549 (pp. 101, 102, 105, 108, 110).

FLEMMING, W., 1882. Zellsubstanz, Kern and Zellteilung. Leipzig, F. C. W. Vogel, 424 pp. (pp. 42, 46, 52, 142).

FUCHS, H., 1901. Ueber das Epithel im Nebenhoden der Maus. Anat. Hefte, 1. Abt., Bd. 19, pp. 313–347 (p. 51).

GALEOTTI, G., 1895. Ueber die Granulationen in den Zellen. Internat. Monatschr. f. Anat. u. Phys., Bd. 12, pp. 461–557 (pp. 52, 60).

GATENBY, J. B., 1917. The cytoplasmic inclusions of the germ-cells. Quart. Jour. Micr. Sci., No. 247, pp. 407–463 (pp. 55, 102).

GERARD, P., 1909. Recherches sur la spermatogenèse chez Stenobothrus biguttulus. Arch. de Biol., t. 24, pp. 543–624 (p. 55).

DE GIACOMO, A., 1911. Sull' ipertrofie compensatoria e sui fenomeni cellulari nei reni, etc. Internat. Monatschr. f. Anat. u. Phys., Bd. 28, pp. 208–232 (p. 137).

GIGLIO-TOS, E., and L. GRANATA, 1908. I. Mitochondrî nelle cellule seminali maschili di Pamphagus marmoratus (Burm). Biologica, vol. 2, No. 4, pp. 1–115 (pp. 45, 50).

GODLEWSKI, E., 1902. Entwickelung des Skelett- und Herzmuskelgewebes der Säugetiere. Arch. f. mikr. Anat., Bd. 60, pp. 111–156 (p. 107).

GOETSCH, EMIL, 1916. Functional significance of mitochondria in toxic thyroid adenomata. Johns Hopkins Hosp. Bull., vol. 27, pp. 129–133 (pp. 44, 100, 117, 135, 136).

GOLDSCHMIDT, R., 1904. Der Chromidialapparat lebhaft funktionierender Gewebszellen. Zool. Jahrb., Abt. f. Anat. u. Ont., Bd. 21, pp. 41–134 (p. 45).

GOLGI, C., 1898. Sur la structure des cellules nerveuses. Arch. Ital. de Biol., t. 30, pp. 60–71 (p. 44).

GOVAERTS, P., 1913. Recherches sur la structure de l' ovaire des insectes. Arch. de Biol., t. 28, pp. 347–444 (pp. 55, 123).

GRYNFELTT, E., 1912a. Sur la présence de chondriosomes dans les cellules de la glande hypobranchiale de Murex trunculus. Bull. Acad. des Sci. et Lettr. Montpellier, pp. 12–17 (p. 51).

———— 1912b. Sur l'appareil mitochondrial des cellules glandulaires de la glande hypobranchiale de Murex trunculus. C. rend. Soc. Biol., t. 72, pp. 261–263 (pp. 55, 147).

———— 1912c. Note sur le chondriome des cellules épithéliales de la glande thyroïde. Bull. Acad. des Sci. et Lettr. Montpellier, pp. 143–148 (pp. 102, 116).

———— 1913. Sur la genèse des boules piriphiles dans la glande hypobranchiale de Murex trunculus. Bull. Acad. des Sci. et Lettr. Montpellier, p. 11 (pp. 102, 120).

GRYNFELTT and EUZIÉRE, 1912. Recherches cytologiques sur les cellules épithéliales des plexus choroïdes de quelques mammifères. C. rend. Assoc. Anat., p. 64 (pp. 101, 117).

———— 1913a. Recherches sur les variations fonctionnelles du chondriome des cellules des plexus choroïdes chez quelques mammifères. C. rend. Assoc. Anat., pp. 197–205 (p. 118).

———— 1913b. Note sur la structure de l'épithélium des toiles choroïdiennes et l'excrétion du liquide céphalo-rachidien chez le scyllium. C. rend. Assoc. Anat., pp. 101–111 (pp. 56, 118).

GUIEYSSE-PELISSIER, A., 1910. Étude des mitochondries de l'organe entérique des crustacés décapodes. Bibl. Anat., t. 20, pp. 18–24 (pp. 51, 54).

GUILLIERMOND, A., 1912a. Recherches cytologiques sur le mode de formation de l'amidon et sur les plastes végétaux, etc. Arch. d'anat. micr., t. 14, pp. 309–428 (pp. 53, 57, 58, 78, 97, 102, 114).

————1912b. Sur le mode de formation du pigment dans la racine de carotte. C. rend. Acad. Sci., t. 155, pp. 411–414 (p. 57).

———— 1912c. Sur les mitochondries des organes sexuels des végétaux. C. rend. Acad. Sci., t. 154, pp. 888–891 (p. 57).

———— 1912d. Nouvelles remarques sur l'origine des chlorleucites. C. rend. Soc. Biol., t. 73, p. 86 (p. 57).

———— 1913a. Sur les mitochondries des champignons. C. rend. Soc. Biol., t. 74, pp. 618–621 (pp. 52, 56).

———— 1913b. Nouvelles observations sur le chondriome de l'asque de Pustularia vesiculosa. C. rend. Soc. Biol., t. 75, pp. 646–649 (pp. 52, 56).

GUILLIERMOND, A., 1913c. Quelques remarques nouvelles sur la formation des pigments anthocyaniques au sein des mitochondries. C. rend. Soc. Biol., t. 75, pp. 478–481 (p. 101).

————1913d. Nouvelles remarques sur la signification des plastes de W. Schimper. C. rend. Soc. Biol., t. 75, pp. 437–440 (pp. 57, 102).

———— 1913e. Sur le rôle du chondriosome dans l' élaboration des produits de réserve des champignons. C. rend. Acad. Sci., t. 157, pp. 63–65 (p. 56).

———— 1913f. Nouvelles recherches cytologiques sur la formation des pigments anthocyaniques. C. rend. Acad. Sci., t. 157, pp. 1000–1002 (pp. 57, 101, 102).

———— 1913g. Nouvelles observations sur le chondriome des champignons. C. rend. Acad. Sci., t. 156, pp. 1781–1784 (p. 56).

———— 1913h. Sur la formation de l'anthocyane au sein des mitochondries. C. rend. Acad. Sci., t. 156, pp. 1924–1926 (p. 102).

———— 1913i. Sur l'étude vitale du chondriome de l' épiderme des pétales d'Iris germanica et de son évolution en leuco- et chromoplastes. C. rend. Soc. Biol., t. 74, pp. 1280–1283 (p. 102).

———— 1914a. Nouvelles remarques sur les plastes végétaux. Anat. Anz., Bd. 46, pp. 566–574 (pp. 53, 57).

———— 1914b. État actuel de la question de l'évolution et du rôle physiologique des mitochondries. Rev. gén. de Botanique, t. 26, pp. 129–149; 182–208 (pp. 52, 53, 56, 57).

———— 1915. Nouvelles observations vitales sur le chondriome des cellules épidermiques de la fleur d'Iris germanica. C. rend. Soc. Biol., t. 78, pp. 241–245 (p. 57).

———— 1917. Recherches sur l'origine des chromoplastes et le mode de formation des pigments du groupe des xanthophylles et carotines. C. rend. Acad. Sci., t. 164, pp. 232–235 (pp. 53, 57).

GUREWITSCH, A., 1913. Vorlesungen über allgemeine Histologie. Jena, Gustav Fischer, 345 pp. (pp. 107, 110).

HALLIBURTON, W. D., 1907. Die Biochemie der peripheren Nerven. Ergebn. d. Phys., Bd. 4, pp. 1–88 (p. 138).

HEGNER, R. W., 1914. Studies on germ cells. I. The history of the germ cells in insects with special reference to the Keimbahn-determinants in animals. Jour. Morph., vol. 25, pp. 375–510 (pp. 55, 102).

HEIDENHAIN, R., 1874. Mikroskopische Beiträge zur Anatomie und Physiologie der Nieren. Arch. f. mikr. Anat., Bd. 10, pp. 1–50 (pp. 44, 51).

HEIDENHAIN, M., 1900. Die Centralkapseln und Pseudochromosomen in den Samenzellen von Proteus, sowie über ihr Verhältnis zu den Idiozomen, Chondromiten und Archoplasmaschleifen usw. Anat. Anz., Bd. 18, pp. 513–550 (pp. 44, 46).

———— 1911. Plasma und Zelle. Jena, Gustav Fischer, 1110 p. (p. 107).

HELD, H., 1895. Beiträge zur Struktur der Nervenzellen und ihrer Fortsätze. Erste Abhandlung. Arch. f. Anat. u. Phys., Anat. Abt., pp. 396–416 (p. 46).

———— 1912. Ueber den Vorgang der Befruchtung bei Ascaris megalocephala. Verh. d. anat. Ges., pp. 242–248 (p. 95).

HENNEGUY, 1904. Les Insectes, Paris (quoted from Duesberg, 1912, p. 576) (p. 55).

HENSCHEN, F., 1904. Zur Struktur der Eizelle gewisser Crustaceen und Gastropoden. Anat. Anz., Bd. 24, pp. 15–29 (p. 54).

HERMANN, F., 1891. Beitrag zur Lehre von der Entstehung der karyokinetischen Spindel. Arch. f. mikr. Anat., Bd. 37, pp. 569–586 (p. 44).

HERTWIG, 1893. Die Zelle und die Gewebe. Jena, Gustav Fischer, 296 pp. (p. 46).

HIRSCH, C., 1910. Experimentell-anatomische Untersuchungen an der Nierenzelle. Anat. Hefte, 1. Abt., Bd. 41, pp. 131–172 (p. 137).

HIRSCHLER, J., 1913. Ueber die Plasmastrukturen (Mitochondrien, Golgischer Apparat u. a.) in den Geschlechtszellen der Ascariden. Arch. f. Zellforsch, Bd. 9, pp. 351–398 (p. 54).

———— 1914. Ueber Plasmastrukturen (Golgischer Apparat, Mitochondrien u. a.) in den Tunicaten-, Spongien- und Protozoenzellen. Anat. Anz., Bd. 47, pp. 289–311 (p. 53).

HJELT, K. J., 1912. Ueber die Mitochondria in den Epithelzellen der gewundenen Nierenkanälchen bei der Einwirkung einiger Diuretika (Kaffein und Theocin). Virchow's Arch., Bd. 207, pp. 297–313 (p. 137).

D'HOLLANDER, F., 1902. Le noyau vitellin de Balbiani et les pseudochromosomes chez les oiseaux. Verh. d. anat. Ges., pp. 168–171 (p. 56).

HOLMGREN, E., 1899. Zur Kenntnis der Spinalganglionzellen von Lophius piscatorius, Lin. Anat. Hefte, 1. Abt., Bd. 12, pp. 75–151 (p. 46).

———— 1903. Ueber die "Saftkanälchen" der Leberzellen und der Epithelzellen der Nebenniere. Anat. Anz., Bd. 22, pp. 9–14 (p. 46).

———— 1909. Studien über die stofflichen Veränderungen der quergestreiften Muskelfasern. Skandin. Arch. f. Phys., Bd. 21, pp. 287–314 (pp. 55, 130, 132).

HOLMGREN, N., 1902. Ueber den Bau der Hoden und die Spermatogenese von Silpha carinata. Anat. Anz., Bd. 22, (p. 55).

HOMANS, J., 1915. A study of experimental diabetes in the canine and its relation to human diabetes. Jour. Med. Res., vol. 33, pp. 1–51 (p. 132, 135).

HOVEN, H., 1910a. Sur l'histogenèse du système nerveuse périphérique, etc. Arch. de Biol., t. 25, pp. 427–491 (pp. 101, 107, 110).

———— 1910b. Contribution à l'étude du fonctionnement des cellules glandulaires. Anat. Anz., Bd. 37, pp. 343–351 (pp. 102, 120).

———— 1911. Du rôle du chondriome dans l'élaboration des produits de sécrétion de la glande mammaire. Anat. Anz., Bd. 39, pp. 321–326 (pp. 51, 102, 119).

HWOROSTUCHIN, W., 1911. Zur Frage über den Bau des Plexus chorioideus. Arch. f. mikr. Anat., Bd. 77, pp. 232–244 (pp. 52, 117).

ISRAEL, O., 1891. Die anämische Nekrose der Nierenepithelien. Virchow's Archiv, Bd. 123, pp. 310–334 (pp. 136, 147).

JENKINSON, J. W., 1914. On the relation between the structure and the development of the centrifuged egg. Quart. Jour. Micr. Sci., vol. 60, pp. 61–158 (p. 96).

JORDAN, H. E., 1911. The spermatogenesis of the opossum (Didelphys virginiana), with special reference to the accessory chromosome and the chondriosomes. Arch. f. Zellforsch., Bd. 4, pp. 41–86 (p. 56).

————, and FERGUSON. 1916. A text-book of histology. New York, D. Appleton & Co., 799 pp. (pp. 46, 107).

JOST, L., 1907. Lectures in plant physiology, translated. Clarendon Press. (p. 145).

DE KERVILY, M., 1916. Le chondriome des cellules de Langhans du placenta humain. C. rend. Soc. Biol., t. 79, pp. 589–590 (p. 51).

KEY, J. A., 1916. On the relation of mitochondria to zymogen granules. Anat. Rec., vol. 10, pp. 215–216 (p. 121).

KINGSBURY, B. F., 1911. The histological demonstration of lipoids. Anat. Rec., vol. 5, pp. 313–318 (p. 63).

———— 1912. Cytoplasmic fixation. Anat. Rec., vol. 6, pp. 39–49 (pp. 75, 133).

KLEIN, S., 1910. Ueber die sog. Schriddeschen Granula. Folia hæm., Bd. 9, t. 11, pp. 406–410 (pp. 52, 135).

KOLLMANN, M., and L. PAPIN, 1914. Étude sur la kératinisation, l'épithélium corné de l'œsophage de quelques mammifères. Arch. d'Anat. micr., t. 16, pp. 193–260 (p. 51).

KOLTZOFF, N. K., 1906. Studien über die Gestalt der Zelle. I. Untersuchungen über die Spermien der Decapoden als Einleitung in das Problem der Zellengestalt. Arch. f. mikr. Anat., Bd. 67, pp. 364–571 (pp. 46, 48, 54, 63).

KOPSCH, F., 1902. Die Darstellung des Binnennetzes in Spinal-Ganglienzellen und anderen Körperzellen mittelst Osmiumsäure. Sitzungsb. d. k. preuss. Akad. d. Wiss., Bd. 40, pp. 929–935 (p. 44).

KOROTNEFF, A., 1909. Mitochondrien, Chondriomiten und Faserepithel der Tricladen. Arch. f. mikr. Anat., Bd. 74, pp. 1000–1016 (pp. 54, 86).

KÖSTER, H., 1910. Morphologie und Genese der Spermatozoen von Gammarus pulex. Zool. Anz., Bd. 35, pp. 490–496 (p. 54).

KROGH, A., 1916. The respiratory exchange of animals and man. Monographs on Biochemistry, London, 173 pp. (p. 146).

KULL, H., 1913. Eine Modification der Altmannschen Methode zum Färben der Chondriosomen. Anat. Anz., Bd. 45, pp. 153–157 (p. 60).

KUSCHAKEWITSCH, S., 1911. Ueber die Entwicklung der Spermien bei Conus mediterraneus Brug. und Vermetus gigas, Biv. Biol. Centralbl., Bd. 31, pp. 530–537 (p. 55).

LABBÉ, A., 1904. La maturation des spermatides et la constitution des spermatozoïdes chez les Crustacés décapodes. Arch. Zool. Exp., vol. 11, Notes et Revues, pp. i–xiv (p. 54).

LAGUESSE, E., 1900. Sur les paranuclei et le mécanisme probable de l'élaboration dans la cellule pancréatique de la salamandre. 13th Cong. Internat. de Méd., Sect. Hist. et Embr., Paris, pp. 1–9 (p. 46).

———— 1911. Ergastoplasme et chondriome dans les cellules sécrétantes séreuses. Bibl. Anat., t. 21, pp. 273–286 (pp. 45, 121).

———— 1912a. Méthode de coloration vitale des chondriosomes par le vert Janus. C. rend. Soc. Biol., t. 73, pp. 150–153.

———— 1912b. Sur l'apparition de la substance amorphe et des premières fibrilles dans les tendons. C. rend. Assoc. Anat., pp. 110–112 (p. 135).

LAMS, H., 1908. Les divisions des spermatocytes chez la fourmi (Camponotus herculeanus). Arch. f. Zellforsch., Bd. 1, pp. 528–537 (p. 55).

———— 1910. Recherches sur l'œuf d'Arion empiricorum (Fér). Acad. roy. de Belg. Classe d. sci. Mém., 144 pp. (p. 55).

LEBOUCQ, G., 1909. Contribution a l'étude de l'histogenèse de la rétine chez les Mammifères. Arch. d'Anat. micr., t. 10, pp. 555–605 (pp. 52, 124, 125).

LEPLAT, G., 1910. La spermiogenèse chez le chat. Arch. de Biol., t. 25, pp. 401–426.

—— 1912. Recherches sur le développement et la structure de la membrane vasculaire de l'œil des oiseaux. Arch. de Biol., t. 27, pp. 403–524 (p. 107).

—— 1913. Les plastosomes des cellules visuelles et leur rôle dans le différenciation des cones et des bâtonnets. Anat. Anz., Bd. 45, pp. 215–221 (pp. 102, 125).

LE TOUZE, M. H., 1912. Contribution a l'étude histologique des Fucacées. Rev. gén. de Botanique, t. 24, pp. 33 (pp. 53, 56, 102).

LEVI, G., 1896. Contributo alla fisiologia della cellula nervosa. Riv. di Path. Nerv. e Ment., vol. 1, pp. 169–180 (p. 101).

—— 1911. Sulla presunta partecipazione dei condriosomi alla differenziazione cellulare. Arch. ital. di Anat. e di Embriol., vol. 10, pp. 168–195 (pp. 107, 110).

—— 1912. I condriosomi nelle cellule secernenti. Anat. Anz., Bd. 42, pp. 576–592 (p. 56).

—— 1913. Note citologiche sulle cellule somatiche dell' ovaio dei Mammiferi. Arch. f. Zellforsch., Bd. 11, pp. 515–556 (pp. 51, 56).

—— 1914. Ulteriori studî sullo sviluppo delle cellule visive negli Anfibi. Anat. Anz., Bd. 47, pp. 192–199 (p. 126).

—— 1915. Il comportamento dei condriosomi durante i più precoci periodi dello sviluppo dei Mammiferi. Arch. f. Zellforsch., Bd. 13, pp. 471–524 (p. 95).

—— 1916a. La costituzione del protoplasma studiata su cellule viventi coltivate in vitro. Arch. d. Fisiologia, vol. 16, pp. 101–112 (pp. 52, 58).

—— 1916b. Il ritmo e le modalità dellá mitosi nelle cellule viventi coltivate "in vitro." Arch. ital. d. Anat. e d Embriol., vol. 15, pp. 243–264.

—— 1916c. Differenziazione "in vitro" di fibre da cellule mesenchimali e loro accrescimento per movimento ameboide. Monit. zool. ital., pp. 77–84 (p. 108).

LEWIS, M. R., 1917. Development of connective-tissue fibers in tissue cultures of chick embryos. Carnegie Inst. of Wash. Contributions to Embryology, No. 17, pp. 45–60 (p. 105).

LEWIS, M. R., and W. H., 1914. Mitochondria in tissue cultures. Science, n. s., vol. 39, pp. 330–333 (pp. 43, 53).

—— 1915. Mitochondria (and other cytoplasmic structures) in tissue cultures. Amer. Jour. Anat., vol. 17, pp. 339–401 (pp. 45, 47, 58, 64, 68, 70, 76, 79, 134).

LEWIS, M. R., and W. R. B. ROBERTSON, 1916. The mitochondria and other structures observed by the tissue culture method in the male germ-cells of Chorthippus curtipennis, Scudd. Biol. Bull., vol. 30, pp. 99–125 (p. 55).

LEWITSKY, G., 1910. Ueber die Chondriosomen in pflanzlichen Zellen. Ber. d. deutsch. bot. Ges., Bd. 28, pp. 538–546 (pp. 53, 57, 102, 107, 114).

—— 1911. Die Chloroplastenanlagen in lebenden und fixierten Zellen von Elodea canadensis. Ber. d. deutsch. bot. Ges., Bd. 29, pp. 697–703 (p. 57).

—— 1913. Die Chondriosomen als Secretbildner bei den Pilzen. Ber. d. deutsch. bot. Ges., Bd. 31, pp. 517–528 (pp. 52, 56).

LILLIE, F. R., 1908. A contribution towards an experimental analysis of the karyokinetic figure. Science, n. s., vol. 27, pp. 907–908 (p. 74).

LILLIE, F. R., 1912. Studies of fertilization in Nereis, III and IV. Jour. Exper. Zool., vol. 12, pp. 413–477 (p. 97).

LILLIE, R. S., 1913. The formation of indophenol at the nuclear and plasma membranes of frog's blood corpuscles and its acceleration by induction shocks. Jour. Biol. Chem., vol. 15, pp. 237–248 (p. 146).

LOBENHOFFER, W., 1906. Ueber die Ergebnisse der Altmann-Schriddeschen Färbenmethode beim Zentralnervensystem. Arch. f. mikr. Anat., Bd. 68, pp. 491–500 (p. 101).

LOEB, J., 1916. The sex of parthenogenetic frogs. Proc. Nat. Acad. Sci., vol. 2, pp. 313–317 (p. 95).

LÖWSCHIN, A. M., 1913. Myelinformen und Chondriosomen. Ber. d. deutsch. bot. Ges., Bd. 31, pp. 203–209 (p. 83).

—— 1914. Vergleichende experimental-cytologische Untersuchungen, etc. Ber. d. deutsch. bot. Ges., Bd. 32, pp. 266–270 (pp. 56, 57, 83).

LOYEZ, M., 1909. Les premiers stades de la vitellogenèse chez quelques Tuniciers. C. rend. Assoc. Anat., pp. 189–195 (pp. 55, 76, 102, 123).

LUBARSCH, O., 1897. Fettdegeneration und Fettinfiltration. Ergebn. d. allg. Path. u. path. Anat., Bd. 3, pp. 631–651 (pp. 135, 137).

LUNA, E., 1913a. Lo sviluppo dei plastosomi negli anfibi. Anat. Anz., Bd. 45, pp. 19–21.

—— 1913b. Nuove ricerche sulla biologia del condrioma (Condriosomi e pigmento retinico). Anat. Anz., Bd. 43, pp. 56–58 (p. 102).

—— 1913c. Sulla importanza dei condriosomi nella genesi delle miofibrille. Arch. f. Zellforsch., Bd. 9, pp. 458–478 (p. 107).

—— 1913d. Ricerche sulla biologia dei condriosomi. Condriosomi e pigmento retinico. Arch. f. Zellforsch., Bd. 10, pp. 343–358.

—— 1913e. I condriosomi nelle cellule nervose. Nota preventiva. Anat. Anz., Bd. 44, pp. 142–144.

—— 1913f. Sui fenomeni di plastorexi e di plastolisi riscontrabili nel processo di involuzione del pronefro negli Anfibi. Nota preventiva. Monit. zool. ital., pp. 131–133 (p. 51).

—— 1913g. Sulle modificazioni dei plastosomi delle cellule nervose nel trapianto ed in seguito al taglio dei nervi. Anat. Anz., Bd. 44, pp. 413–415 (p. 137).

MACALLUM, A. B., 1908. Die Methoden und Ergebnisse der Mikrochemie in der biologischen Forschung. Ergebn. d. Phys., Bd. 7, pp. 5 2–652 (p. 145).

MAGGI, L., 1878. I plastiduli nei ciliati ed i plastiduli liberamente viventi. Atti. Soc. Sci. Nat., Milano, t. 21, pp. 326–330 (p. 42).

MAGGIORE, L., 1912. L'apparato mitochondriale nel cristallino. Ricerche Lab. di Anat. norm. Univ. Roma, vol. 16, pp. 117–119 (p. 52).

MANCA, P., 1913. Sulla presenza di condrioconti nelle cellule degli abbozzi dentarii. Nota preliminare. Monit. zool. ital., pp. 121–127 (p. 51).

MARCORA, F., 1911. Ueber die Histogenese des Zentralnervensystems mit besonderer Rücksicht auf die innere Struktur der Nervenelemente. Folia neuro-biol., Bd. 5, pp. 928–960 (p. 110).

MATHEWS, A. P., 1905. A theory of the nature of protoplasmic respiration and growth. Biol. Bull., vol. 8, pp. 331–346 (p. 147).

—— 1915. Physiological chemistry. 1040 pp. New York, William Wood & Co. (pp. 71, 129, 134, 139, 144).

MAWAS, J., 1909. La structure de la rétine ciliaire et la sécrétion de l'humeur aqueuse. C. rend. Assoc. Anat., pp. 282–285 (p. 101).

—— 1910a. Sur la structure des cellules nerveuses ganglionnaires de la moelle amyélinique des Cyclostomes. C. rend. Acad. des Sci., t. 150, pp. 126–127 (p. 55).

—— 1910b. Étude cytologique et physiologique sur la rétine ciliaire des Mammifères. Arch. d'Anat. micr., t. 12, pp. 103–176 (pp. 124, 125).

—— 1910c. Notes cytologiques sur les cellules visuelles de l'homme et de quelques mammifères. C. rend. Assoc. Anat., pp. 113–118 (p. 125).

MAXIMOW, A., 1913. Ueber Chondriosomen in lebenden Pflanzenzellen. Anat. Anz., Bd. 43, pp. 241–249 (pp. 53, 57).

—— 1916a. Sur les méthodes de fixation et de coloration des chondriosomes. C. rend. Soc. Biol., t. 79, pp. 462–463 (p. 63).

—— 1916b. Sur la structure des chondriosomes. C. rend. Soc. Biol., t. 79, pp. 465–466 (p. 52).

MAYER, A. and RATHERY, 1907. Études sur le corps fungiforme du poulpe. Jour. de l'Anat. et de Phys., t. 43, pp. 25–47 (p. 55).

—— 1909. Histophysiologie du rein. Jour. de l' Anat. et de Phys., t. 45, pp. 321–338 (p. 54).

MAYER, A., RATHERY and SCHAEFFER, 1914. Les granulations ou mitochondries de la cellule hépatique. Deuxième Partie. Jour. de Phys. et de Path. gén., t. 16, pp. 607–622 (pp. 84, 134, 135).

MAZIARSKI, S., 1903. Recherches cytologiques sur les organes segmentaries des vers de terre. Arch. polon. sci. biol. et méd., t. 2, pp. 1–81 (p. 54).

McCANN, GERTRUDE FISHER, 1918. A study of mitochondria in experimental poliomyelitis. Jour. Exp. Med., vol. 27, pp. 31–36 (p. 137).

McCLENDON, J. F., 1910. Further studies on the gametogenesis of Pandarus sinuatus. Arch. f. Zellforsch., Bd. 5, pp. 229–234 (p. 54).

METZNER, R., 1890. Ueber die Beziehungen der Granula zum Fettansatz. Arch. f. Anat. u. Phys., Anat. Abt., pp. 82–96 (pp. 52, 124).

MEVES, F., 1897. Zellteilung. Ergebn. d. Anat. u. Entw. (Anat. Hefte, 2. Abt.), Bd. 6, pp. 284–397 (p. 45).

—— 1900. Ueber den von la Valette St. George entdeckten Nebenkern (Mitochondrienkörper) der Samenzellen. Arch. f. mikr. Anat., Bd. 56, pp. 552–606 (pp. 55, 102).

—— 1904. Ueber das Vorkommen von Mitochondrien bezw. Chondriomiten in Pflanzenzellen. Ber. d. deutsch. bot. Ges., Bd 22, pp. 284–286 (pp. 53, 57).

—— 1905. Ueber die Wirkung gefärbter Jodsäure auf die roten Blutkörperchen der Amphibien. Anat. Anz., Bd. 26, pp. 97–103 (pp. 63, 102, 126).

—— 1907a. Ueber Mitochondrien bezw. Chondriokonten in den Zellen junger Embryonen. Anat. Anz., Bd. 31, pp. 399–407 (pp. 44, 45, 47, 52, 56, 102, 105, 110, 113).

—— 1907b. Die Chondriokonten in ihrem Verhältnis zur Filarmasse Flemmings. Anat. Anz., Bd. 31, pp. 561–569 (p. 51).

—— 1907c. Die Spermatocytenteilungen bei der Honigbiene (Apis mellifica L.), nebst Bemerkungen über Chromatinreduktion. Arch. f. mikr. Anat., Bd. 70, pp. 414–491 (p. 55).

—— 1908. Die Chondriosomen als Träger erblicher Anlagen. Cytologische Studien am Hühnerembryo. Arch. f. mikr. Anat., Bd. 72, pp. 816–867 (pp. 43, 48, 96, 101, 110, 129).

MEVES, F., 1910a. Ueber Strukturen in den Zellen des embryonalen Stützgewebes sowie über die Entstehung der Bindegewebsfibrillen, insbesonders derjenigen der Sehne. Arch. f. mikr. Anat., Bd. 75, pp. 149–208 (pp. 46, 48, 102, 103).

—— 1910b. Zur Einigung zwischen Faden- und Granulalehre des Protoplasma. Beobachtungen an weissen Blutzellen. Arch. f. mikr. Anat., Bd. 75, pp. 642–658 (pp. 101, 102, 110, 126).

—— 1911a. Gesammelte Studien an den roten Blutkörperchen der Amphibien. Arch. f. mikr. Anat., Bd. 28, pp. 465–540 (pp. 52, 105, 115).

—— 1911b. Ueber die Beteiligung der Plastochondrien an der Befruchtung des Eies von Ascaris megalocephala. Arch. f. mikr. Anat., Bd. 76, pp. 683–714 (p. 95).

—— 1912. Verfolgung des sogenannten Mittelstückes des Echinidienspermiens im befruchteten Ei bis zum Ende der ersten Furchungteilung. Arch. f. mikr. Anat., Bd. 80, pp. 81–123 (p. 95).

—— 1913. Ueber das Verhalten des plastomatischen Bestandteiles bei der Befruchtung des Eies von Phallusia mammillata. Arch. f. mikr. Anat., Bd. 82, pp. 215–260 (pp. 55, 95).

—— 1915a. Was sind die Plastosomen? II. Bemerkungen zu dem Vortrag von C. Benda. Die Bedeutung der Zelleibstruktur für die Pathologie. Arch. f. mikr. Anat., Bd. 87, pp. 287–308.

—— 1915b. Ueber Mitwirkung der Plastosomen bei der Befruchtung des Eies von Filaria papillosa. Arch. f. mikr. Anat., Bd. 87, 2. Abt., pp. 12–46 (pp. 54, 95).

—— 1915c. Ueber den Befruchtungsvorgang bei der Miesmuschel (Mytilus edulis L.). Arch. f. mikr. Anat., Bd. 87, 2. Abt., pp 47–62 (p. 95).

MEVES, F. and J. DUESBERG, 1908. Die Spermatocytenteilungen bei der Hornisse (Vespa crabro L.). Arch. f. mikr. Anat., Bd. 71, pp. 571–587 (pp. 55, 61, 97).

—— and R. TSUKAGUCHI, 1914. Ueber das Vorkommen von Plastosomen im Epithel von Trachea und Lunge. Anat. Anz., Bd. 46, pp. 289–292.

MICHAELIS, L., 1899. Die vitale Färbung, eine Darstellungsmethode der Zellgranula. Arch. f. mikr. Anat., Bd. 55, pp. 558–575 (pp. 86, 87).

—— 1902. Einführung in die Farbstoffchemie für Histologen. Berlin, S. Karger, 156 pp. (pp. 87, 89).

MIRA, M. FERREIRA, 1912. Sur l'état des capsules surrénales chez les animaux ovariectomisés. Bull. Soc. Portugaise Sci. Nat., t. 6, pp. 34–46 (p. 135).

MIRANDE, M., 1916. Observation sur le vivant de la formation cytologique de l'anthocyanine. C. rend. Acad. Sci., t. 163, pp. 368–371 (p. 56).

MIRONESCO, T., 1912. Le chondriome du réseau de Purkinje du cœur. C. rend. Soc. Biol., t. 72, pp. 30–31 (p. 52).

MISLAWSKY, A. N., 1911a. Demonstration von Chondriosomen in Pankreaszellen. Verh. d. anat. Ges., pp. 221–222 (p. 121).

—— 1911b. Beiträge zur Morphologie der Drüsenzelle. Ueber des Chondriom der Pankreaszelle einiger Nager. Anat. Anz., Bd. 39, pp. 497–505.

MODRAKOWSKI, 1903. Weitere Beiträge zur Nierenfunktion. Pflüger's Arch., Bd. 98, pp. 217–232 (p. 122).

MONTOOMERY, T. H., 1911. The spermatogenesis of an hemipteron, Euschistus. Jour. Morph., vol. 22, pp. 731–821 (p. 55).

MONTI, R., 1915. I condriosomi e gli apparati di Golgi nelle cellule nervose. Arch. ital. di Anat. e di Embriol., vol. 14, pp. 1–45 (p. 63).

MOREAU, F., 1914a. Sur la formation de corpuscules méta-chromatiques dans les mitochondries granuleuses. C. rend. Soc. Biol., t. 77, pp. 347–349 (p. 56).

—— 1914b. Sur le chondriome d'une ustilaginée, Entyloma ranunculi (Bonorden) Schrœter. C. rend. Soc. Biol., t. 77, pp. 538–539 (pp. 56, 67).

—— 1914c. Sur l'origine de l'anthocyane dans les divers organes des végétaux. C. rend. Soc. Biol., t. 77, pp. 502–503 (p. 57).

—— 1914d. Le chondriome et la division des mito-chondries chez les Vaucheria. Bull. de la Soc. bot. de France, t. 61.

—— 1915a. Sur la formation de cristalloides de mucorine au sein des mitochondries. C. rend. Soc. Biol., t. 78, pp. 171–172 (pp. 52, 102).

—— 1915b. La division des mitochondries et ses rapports avec les phénomènes de sécrétion. C. rend. Soc. Biol., t. 78, pp. 143–144 (p. 56).

MOREAU, Mme. F., 1914. Les mitochondries chez les urédinées. C. rend. Soc. Biol., t. 76, pp. 421–422 (pp. 52, 56).

MOREAU, F., and Mme. MOREAU, 1916. Sur le chon-driome d'une algue verte, Coccomyxa solorinae Chod. C. rend. Soc. Biol., t. 79, pp. 211–212 (p. 56).

MOREAUX, R., 1909. Note sur la spermiogenèse du Macaque. C. rend. Soc. Biol., t. 67, pp. 369–371 (p. 56).

MULON, P., 1910a. La méthode des mitochondries (de Benda) appliquée à la corticale surrénale du cobaye. C. rend. Soc. Biol., t. 68, pp. 103–105 (p. 52).

—— 1910b. Sur les mitochondries de la surrénale. C. rend. Soc. Biol., t. 68, pp. 872–873 (p. 102).

—— 1912. Note sur la capsule surrenale du Mouton. Bibl. Anat., t. 22, pp. 30–36 (p. 69).

—— 1913. Du rôle des lipoides dans la pigmentogenèse. C. rend. Soc. Biol., t. 74, pp. 1023–1027 (p. 102).

MUNSON, J. P., 1898. The ovarian egg of Limulus. Jour. Morph., vol. 15, pp. 111–211 (p. 55).

—— 1904. Researches on the oogenesis of the tortoise, Clemmys marmorata. Amer. Jour. Anat., vol. 3, pp. 311–348 (p. 56).

NAEGELI, O., 1912. Blutkrankheiten und Blutdiag-nostik. Leipzig, Veit & Co., 719 pp. (p. 52).

NAGEOTTE, J., 1909. Mitochondries du tissu nerveux. C. rend. Soc. Biol., t. 66, pp. 825–828 (pp. 52, 101).

NELIS, C., 1899. Un nouveau détail de structure du protoplasme des cellules nerveuses (état spiré-mateux du protoplasme). Bull. de la classe des Sci. de l'Acad. Belg., pp. 102–125 (p. 46).

NÉMEC, B., 1910. Das Problem der Befruchtungs-vorgänge und andere zytologische Fragen. Berlin, 532 pp. (pp. 53, 135).

NICHOLSON, N. C., 1916. Morphological and micro-chemical variations in the mitochondria in the cells of the central nervous system. Amer. Jour. Anat., vol. 19, pp. 329–349 (pp. 67, 85, 131, 148).

NICOLAS, J., CL. REGAUD, and M. FAVRE, 1912a. Sur les mitochondries des glandes sébacées de l'homme et sur la signification générale de ces organites du protoplasma. C. rend. Assoc. Anat., pp. 201–205 (pp. 51, 73, 102, 119).

NICOLAS, J., CL. REGAUD, and M. FAVRE, 1912b. Sur la fine structure des glandes sudoripares de l'homme particulièrement en ce qui concerne les mitochondries et les phénomènes de sécrétion. C. rend. Assoc. Anat., pp. 191–200 (pp. 51, 102, 119).

NICOLOSI-RONCATI, F., 1910. Formazioni mitochon-driali negli elementi sessuale maschili dell' Helleborus fœtidus. Rend. Acad. Sci. Napoli, Ser. 3, t. 16, pp. 109–119.

—— 1912a. Formazioni endocellulari nelle Rodo-ficee. Bull. Soc. Bot. Ital., pp. 59–62 (p. 56).

—— 1912b. Genesi dei cromatofori nelle Fucoidee. Bull. Soc. Bot. Ital., pp. 144–149 (pp. 53, 56, 102).

—— 1912c. Contributo alla conoscenza cito-fisio-logica delle glandule vegetali. Bull. Soc. Bot. Ital., pp. 186–193 (p. 57).

NORDENSKIÖLD, E., 1909. Zur Spermatogenese von Ixodes reduvius. Zool. Anz., Bd. 34, pp. 511–516 (p. 55).

OETTINGER, R., 1909. Zur Kenntnis der Spermato-genese bei den Myriopoden: Samenreifung und Samenbildung bei Pachyiulus varius, Fabre. Arch. f. Zellforsch., Bd. 3, pp. 563–626 (p. 55).

OLIVER, J., 1916. A further study of the regenerated epithelium in chronic uranium nephritis. An anatomical investigation of its function. Jour. Exper. Med., vol. 23, pp. 301–322 (pp. 102, 122, 135, 139).

OPHÜLS, W., 1907. Altmann's granules in granular and fatty degeneration in the kidney. Proc. Soc. Exper. Biol. and Med., vol. 4, pp. 135–137 (p. 135).

ORMAN, E., 1912. Recherches sur les différenciations cytoplasmiques (ergastoplasme et chondriosomes) dans les végétaux. I. Le sac embryonnaire des Liliacées. Cellule, t. 28, pp. 363–444 (p. 57).

PANTEL, J., and R. DE SINÉTY, 1906. Les cellules de la lignée mâle chez le Notonecta glauca. Cellule, t. 23, pp. 90–303 (p. 55).

PARKER, T. J., and W. A. HASWELL, 1897. Text-book of Zoology, vol. 1. London, Macmillan & Co., 779 pp. (p. 145).

PAYNE, F., 1916. A study of the germ cells of Gryllo-talpa borealis and Gryllotalpa vulgaris. Jour. Morph., vol. 28, pp. 287–327 (p. 55).

PENSA, A., 1910. Alcune formazioni endocellulari dei vegetali. Anat. Anz., Bd. 37, pp. 325–333 (pp. 57, 63, 114).

—— 1911. Ancora di alcune formazioni endocellulari dei vegetali. Anat. Anz., Bd. 39, pp. 520–532 (pp. 53, 56, 57).

—— 1912. Osservazioni di morfologia e biologia cellulare nei vegetali (mitocondri, cloroplasti). Arch. f. Zellforsch., Bd. 8, pp. 612–662 (pp. 56, 57).

—— 1913a. Condriosomi e pigmento antocianico nelle cellule vegetali. Anat. Anz., Bd. 45, pp. 81–90 (p. 57).

—— 1913b. La struttura della cellula cartilaginea. Arch. f. Zellforsch., Bd. 11, pp. 557–582 (p. 52).

PERRONCITO, A., 1910. Contribution a l'étude de la biologie cellulaire. Arch. ital. de Biol., vol. 54, pp. 307–345 (pp. 44, 45, 102).

PIZZINI, B., 1908. Ueber die Sekretionserscheinungen in der Nierenzelle bei der Diurese. Internat. Monatsschr. f. Anat. u. Phys., Bd. 25, pp. 108–148 (p. 136).

PLATNER, G., 1889. Ueber die Bedeutung der Rich-tungskörperchen. Biol. Centralbl., Bd. 8, pp. 718–720 (p. 46).

Policard, A., 1905. Sur les formations mitochondriales du rein des vertébrés. C. rend. Soc. Biol., t. 57, pp. 380–382 (pp. 56, 122).

—— 1910. Contribution a l'étude du mécanisme, etc. Arch. d'Anat. micr., t. 12, pp. 177–288 (pp. 122, 132, 135).

—— 1912a. Rôle du chondriome dans la formation des cristaux intracellulaires de la cellule hépatique. C. rend. Soc. Biol., t. 72, pp. 91–93 (pp. 101, 102).

—— 1912b. Sur le rôle du chondriome dans le formation des cristaux intraprotoplasmique d'hémoglobine dans la cellule hépatique. Bibl. Anat., t. 22, pp. 226–230 (pp. 102, 116).

—— 1912c. La cytogenèse du tube urinaire chez l'homme. Arch. d'Anat. micr., t. 14, pp. 429–468 (pp. 44, 81, 86, 122).

—— 1912d. Sensibilité des chondriosomes aux élévations de température. C. rend. Soc. Biol., t. 72, pp. 228–230 (pp. 83, 139).

—— 1912e. Sur quelques points de la cytologie des plexus choroïdes. C. rend. Soc. Biol., t. 73, pp. 430–432 (p. 117).

—— 1912f. Recherches histophysiologiques sur les premiers stades de la sécrétion urinaire. Arch. d'Anat. micr., t. 14, pp. 1–40 (p. 81).

—— 1914. Recherches histophysiologiques sur les voies biliaires intrahépatiques. Jour. de Phys. et de Path. gén., t. 16, pp. 623–637 (p. 51).

—— 1915. Chondriocontes et fibrilles plasmatiques dans les cellules du tube urinaire des Batraciens. Anat. Anz., Bd. 47, pp. 539–543 (p. 51).

Policard, A., and J. Mawas, 1906. Le canalicule urinaire des Téléostéens. Bibl. Anat., t. 15, pp. 215–221 (p. 56).

Porcelli-Titone, F., 1914. Der Mitochondrienapparat der Geschwulstzellen. Beitr. z. path. Anat. u. allg. Path., Bd. 58, pp. 237–249 (pp. 135, 140).

Prenant, A., 1899. Sur le protoplasma supérieur (archiplasme, kinoplasme, ergastoplasme). Étude critique. Jour. de l'Anat. et de la Phys., t. 35, pp. 408–466 (pp. 45, 46).

—— 1910. Les mitochondries et l'ergastoplasme. Jour. de l'Anat. et de la Phys., t. 46, pp. 217–285 (p. 46).

—— 1911a. Problèmes cytologiques généraux soulevés par l'étude des cellules musculaires. Jour. de l'Anat. et de la Phys., t. 47, pp. 450–524 (p. 107).

—— 1911b. Préparations relatives aux mitochondries. C. rend. Assoc. Anat., pp. 333–337 (p. 51, 52).

—— 1913. Sur l'origine mitochondriale des grains de pigment. C. rend. Soc. Biol., t. 74, pp. 926–929 (p. 102).

Prenant, Bouin and Maillard, 1906. Traité d'Histologie. T. t, 977 pp. Paris, Masson & Cie. (p. 52).

Prowazek, 1902. Ein Beitrag zur Krebsspermatogenese. Zeitschr. f. wiss. Zool., Bd. 71, pp. 445–456 (pp. 54, 55).

Regaud, Cl., 1908a. Sur les formations mitochondriales de diverses espèces cellulaires. C. rend. Assoc. Anat., pp. 15–19 (pp. 51, 56, 81, 145).

—— 1908b. Sur les mitochondries des cellules ciliées du tube urinaire. Ont-elles une relation avec la fonction motrice de ces cellules? C. rend. Soc. Biol., t. 65, pp. 206–208 (p. 130).

—— 1908c. Variations des formations mitochondriales dans les tubes à cuticule striée du rein. C. rend. Soc. Biol., t. 64, pp. 1145–1147.

Regaud, Cl., 1908d. Sur les mitochondries de l'épithélium séminal. C. rend. Soc. Biol., t. 65, pp. 718–720 (pp. 43, 83).

—— 1909a. Attribution aux "formations mitochondriales" de la fonction générale d'extraction et de fixation électives, exercée par les cellules vivantes sur les substances dissoutes dans le milieu ambiant. C. rend. Soc. Biol., t. 66, pp. 919–921 (p. 103).

—— 1909b. Sur les mitochondries des fibres musculaires du cœur. C. rend. Acad. Sc., t. 149, pp. 426–428 (p. 52).

—— 1909c. Participation du chondriomes à la formation des grains de ségrégation dans les cellules des tubes contournés du rein (chez les ophidiens et les amphibiens). C. rend. Soc. Biol., t. 66, pp. 1034–1036 (p. 122).

—— 1910. Étude sur la structure des tubes séminifères, etc. Arch. d'Anat. micr., t. 11, pp. 291–433 (pp. 61, 76, 85, 95, 102).

—— 1911. Les mitochondries. Rev. de Méd., t. 31, pp. 681–699 (pp. 129, 133).

Regaud, Cl., and M. Favre, 1909. Demonstration des mitochondries dans les fibres musculaires striées. C. rend. Assoc. Anat., p. 298 (p. 52).

—— 1912. Nouvelles recherches sur les formations mitochondriales de l'épiderme humain, à l'état normal et pathologique. C. rend. Soc. Biol., t. 72, pp. 328–331 (pp. 105, 135).

Regaud, Cl., and J. Mawas, 1909a. Ergastoplasme et mitochondries dans les cellules de la glande sous-maxillaire de l'homme. C. rend. Soc. Biol., t. 66, pp. 461–463 (p. 45).

—— 1909b. Sur la structure du protoplasma (ergastoplasme, mitochondries, grains de ségrégation) dans les cellules séro-zymogènes des acini et dans les cellules des canaux excréteurs de quelques glandes salivaires de Mammifères. C. rend. Assoc. Anat., pp. 220–237 (pp. 45, 51, 102, 118).

Regaud, Cl., and A. Policard, 1913. Sur la signification de la rétention du chrome par les tissus en technique histologique au point de vue des lipoïdes et des mitochondries. I. Fixation "morphologique" et fixation "de substances." C. rend. Soc. Biol., t. 74, pp. 449–451.

Renaut and Dubreuil, 1906a. Sur les cellules rhagiocrines libres du liquide des diverses séreuses. C. rend. Soc. Biol., t. 60, pp. 34–37.

—— 1906b. Les cellules connectives de la lignée rhagiocrine. Bibl. Anat., t. 15, pp. 222–242 (pp. 45, 46, 102, 127).

Retzius, G., 1914. Was sind die Plastosomen? Arch. f. mikr. Anat., Bd. 84, Abt. 1, pp. 175–214.

Riddle, O., 1916. Sex control and known correlations in pigeons. Amer. Naturalist, vol. 50, pp. 385–410 (p. 84).

Romeis, B., 1912. Beobachtungen über Degenerationserscheinungen von Chondriosomen. Arch. f. mikr. Anat., Bd. 80, pp. 129–170 (p. 44).

—— 1913a. Ueber Plastosomen und andere Zellstrukturen in den Uterus, Darm- und Muskelzellen von Ascaris megalocephala. Anat. Anz., Bd. 44, pp. 1–14 (pp. 51, 108, 138, 147).

—— 1913b. Beobachtungen über die Plastosomen von Ascaris megalocephala während der Embryonalentwicklung unter besonderer Berücksichtigung ihres Verhaltens in den Stamm- und Urgeschlechtszellen. Arch. f. mikr. Anat., Bd. 81, Abt. 2, pp. 129–172 (p. 95).

ROMEIS, B., 1913c. Das Verhalten der Plastosomen bei der Regeneration. Anat. Anz., Bd. 45, pp. 1–19 (pp. 132, 135).

RUBASCHKIN, W., 1910. Chondriosomen und Differenzierungsprozesse bei Säugetierembryonen. Anat. Hefte, 1. Abt., Bd. 41, pp. 399–431 (pp. 63, 67).

RUDOLPH, K., 1912. Chondriosomen und Chromatophoren. Beitrag zur Kritik der Chondriosomentheorien. Ber. d. deutsch. bot. Ges., Bd. 30, pp. 605–629 (pp. 53, 56, 57).

RUSSO, A., 1910. Sui mutamenti che subiscono i mitochondri ed i materiali deutoplasmici dell' oocite di Coniglia in diversi periodi di inanizione. Arch. f. Zellforsch., Bd. 5, pp. 173–181 (p. 136).

——— 1912. Aumento dei granuli proplasmatici nell' oocite delle Coniglie iniettate con lecitinia, loro diminuzione nelle Coniglie digiunanti e loro natura lipoide e mitochondriale. Arch. f. Zellforsch., Bd. 8, pp. 203–216 (pp. 84, 133).

SAGUCHI, S., 1913. Ueber Mitochondrien (Chondriokonten) und mitochondriale Stränge (=sog. Eberthsche intrazelluläre Gebilde) in den Epidermiszellen der Anurenlarven nebst Bemerkungen über die Frage der Epidermis-Cutis-grenze. Arch. f. mikr. Anat., Bd. 83, Abt. 1, pp. 177–246 (pp. 45, 56, 102, 113).

——— 1915. Ueber Sekretionserscheinungen an den Epidermiszellen von Amphibienlarven nebst Beiträgen zur Frage nach der physiologischen Degeneration der Zellen. Mitteilungen d. med. Fak. d. Univ. z. Tokyo, Bd. 14, 299–415 (p. 102).

——— 1917. Studies on ciliated cells. Jour. Morph., vol. 29, pp. 217–279 (pp. 102, 128).

SAMSSONOW, N., 1910. Ueber die Beziehungen der Filarmasse Flemmings zu den Fäden und Körnern Altmann's nach Beobachtungen an Knorpel-Bindegewebs- und Epidermiszellen. Arch. f. mikr. Anat., Bd. 75, pp. 635–641.

SAPÉHIN, A. A., 1913. Ein Bewiss der Individualität der Plastiden. Ber. d. deutsch. bot. Ges., Bd. 31, pp. 321–324 (p. 53).

——— 1915. Untersuchungen über die Individualität der Plastide. Arch. f. Zellforsch., Bd. 13, pp. 319–398 (pp. 52, 53, 56, 57).

SCHÄFER, E. A. 1912. Text-book of microscopic anatomy. 739 pp. London, Longmans Green & Co. (pp. 46, 55, 107).

SCHÄFER, F., 1907. Spermatogenese von Dytíscus. Zool. Jahrb., Abt. f. Anat., Bd. 23, pp. 535–586.

SCHAXEL, J., 1911. Plasmastrukturen, Chondriosomen und Chromidien. Anat. Anz., Bd. 39, pp. 337–353 (p. 54).

——— 1912. Versuch einer cytologischen Analyse der Entwicklungsvorgänge. I. Die Geschlechtszellenbildung und die normale Entwicklung von Aricia foetida Clap. Zool. Jahrb., Abt. f. Anat. u. Ontog., Bd. 34, pp. 381–472 (p. 54).

SCHERRER, A., 1913. Die Chromatophoren und Chondriosomen von Anthoceros. Ber. d. deutsch. bot. Ges., Bd. 31, pp. 493–500 (pp. 52, 56).

SCHILLING, C., 1894. Das Verhalten der Altmann'schen Granula bei der trüben Schwellung. Virchow's Archiv., Bd. 135, pp. 470–479 (pp. 135, 137).

SCHIROKOGOROFF, J. J., 1913. Die Mitochondrien in den erwachsenen Nervenzellen des Zentralnervensystems. Anat. Anz., Bd. 43, pp. 522–524 (p. 60).

SCHITZ, V., 1916. Sur la spermatogenèse chez Columbella rustica L. Arch. de Zool. exp. et gén., Notes et Revue, t. 56, pp. 32–47 (p. 55).

SCHREINER, A. and K. E., 1905. Ueber die Entwicklung der männlichen Geschlechtszellen von Myxine glutinosa. Arch. de Biol., t. 21, pp. 183–314 (p. 55).

SCHRIDDE, H., 1905. Beiträge zur Lehre von Zellkörnelungen. Die Körnelung der Plasmazellen. Anat. Hefte, 1. Abt., Bd. 28, pp. 691–768 (pp. 52, 60).

——— 1907. Myeloblasten, Lymphoblasten und lymphoblastische Plasmazellen. Ziegler's Beiträge z. path. Anat., Bd. 41, pp. 223–238 (p. 52).

——— 1912. Untersuchungen über die Bildung des Hämoglobins. Anat. Anz., Bd. 42, pp. 514–517 (p. 115).

SCHULTZ, T., 1914. Farbstofftabellen. Berlin, Julius Springer (p. 92).

SCHULTZE, O., 1911a. Ueber die Anwendung der Osmiumsäure und eine neue Osmiumhematoxylin-methode. Zeitschr. f. wiss. Mikr., Bd. 27, pp. 465–475 (p. 63).

——— 1911b. Ueber die Genese der Granula in den Drüsen-Zellen. Anat. Anz., Bd. 38, pp. 257–265 (p. 121).

SCOTT, K. J., 1915. The relation of mitochondria to granules of the vital azo dyes. Science, n. s., vol. 41, pp. 834–835 (p. 58).

SCOTT, W. J. M., 1916. Experimental mitochondrial changes in the pancreas in phosphorus poisoning. Amer. Jour. Anat., vol. 20, pp. 237–253 (pp. 121, 132, 135).

SHIPLEY, P. G., 1915. The mitochondrial substance in the erythrocytes of the embryo pig. Folia hæm., Bd. 20, pp. 61–86 (pp. 56, 135).

——— 1916. The vital staining of mitochondria in Trypanosoma lewisi with janus green. Anat. Rec., vol. 10, pp. 439–446 (pp. 53, 89, 130).

SJOVALL, E., 1906. Ein Versuch, das Binnennetz von Golgi-Kopsch bei der Spermato- und Ovogenese zu homologisieren. Anat. Anz., Bd. 28, pp. 561–579 (pp. 45, 46, 63).

v. SMIRNOW, A. E., 1906. Ueber die Mitochondrien und den Golgischen Bildungen analoge Strukturen in einigen Zellen von Hyacinthas orientalis. Anat. Hefte, 1. Abt., Bd. 32, pp. 146–153 (p. 57).

SMITH, E. F., 1917. Embryomas in plants. Johns Hopkins Hosp. Bull., vol. 28, pp. 277–293 (p. 140).

SOKOLOW, I., 1913. Untersuchungen über die Spermatogenese bei den Arachniden. Arch. f. Zellforsch. Bd. 9, pp. 397–431 (p. 55).

SOLGER, B., 1896. Ueber den feineren Bau der Glandula submaxillaris der Menschen. Festschr. f. C. Gegenbaur, Bd. 2, pp. 180–248 (p. 44).

SPITSCHAKOFF, 1909. Spermien und Spermiohistogenese bei Cariden. Arch. f. Zellforsch., Bd. 3, pp. 1–43 (p. 54).

STRASBURGER, E., 1882. Ueber den Theilungsvorgang der Zellkerne und das Verhältniss der Kerntheilung zur Zelltheilung. Arch. f. mikr. Anat., Bd. 21, pp. 476–590 (p. 45).

STRONOMAN, B. T., 1917. A preliminary experimental study on the relation between mitochondria and discharge of nervous activity. Anat. Rec., vol. 12, pp. 167–171 (p. 131).

SUNDWALL, J., 1916. The lachrymal gland. Amer. Jour. Anat., vol. 20, pp. 147–236 (p. 51).

SWINGLE, W. T., 1898. Two new organs of the plant cell. Bot. Gaz., vol. 25, p. 110 (p. 46).

TAKAKI, K., 1907. Ueber die Stäbchenstruktur der Niere. Arch. f. mikr. Anat., Bd. 70, pp. 245–265 (p. 137).

TASHIRO, S., 1915. On the nature of the nerve-impulse. Proc. Nat. Acad. Sci., vol. 1, pp. 110–114 (p. 74).

THURLOW, M. DEG., 1917. Quantitative studies on mitochondria in nerve-cells. Carnegie Inst. of Wash., Contributions to Embryology, No. 16, pp. 37–44 (pp. 80, 131, 132).

TORRACA, L., 1914a. Il comportamento dei condriosomi nella rigenerazione dei muscoli striati. Arch. f. Zellforsch., Bd. 12, pp. 539–552 (pp. 135, 138).

———— 1914b. Alcune osservazioni sui condriosomi delle cellule cartilaginee nella coda del tritone rigenerante. Anat. Anz., Bd. 45, pp. 459–474 (pp. 135, 138).

———— 1916. Alcune osservazioni sulla rigenerazione e sul processo di secrezione delle glandole velogene delle Triton cristatus. Arch. ital. di Anat. e di Embriol., vol. 15, pp. 283–330 (pp. 102, 138).

TSCHASCHIN, S., 1912. Ueber vitale Färbung der Chondriosomen in Bindegewebszellen mit Pyrolblau. Folia hæm., Bd. 14, pp. 295–307 (p. 58).

TSUKAGUCHI, R., 1914. Ueber die feinere Struktur des Ovarialeies von Aurelia aurita L. Arch. f. mikr. Anat., Bd. 85, Abt. II, pp. 114–123 (p. 51, 54, 99).

VALETTE ST. GEORGE, A., 1887. Zellteilung und Samenbildung bei Forficula auricularia. Festschr. f. Koelliker (p. 55).

VAN DER STRICHT, O., 1898. Contribution à l'étude du noyau vitellin de Balbiani dans l'oocyte de la femme. Verh. d. anat. Ges., pp. 128–139 (p. 55).

———— 1902. Les "pseudochromosomes" dans l'oocyte de chauve-souris (V. noctula). C. rend. Assoc. Anat., pp. 1–60.

———— 1904. La couche vitellogène et les mitochondries de l'œuf des Mammifères. Verh. d. anat. Ges., pp. 138–145 (p. 45).

———— 1905. La structure de l'œuf de chauve-souris (V. noctula). C. rend. Assoc. Anat., pp. 6–12 (p. 51).

———— 1908. La structure de l'œuf de chienne et la genèse du corps jaune. C. rend. Assoc. Anat., pp. 1–9 (pp. 56, 96).

———— 1909. La structure de l'œuf des mammifères (chauve-souris, Vesperugo noctula). Troisième partie. Acad. roy. Belgique. Classe des sci. Mém. 2e. série, t. 2, 173 pp. (p. 76).

———— 1918. The genesis and structure of the membrana tectoria and the crista spiralis of the cochlea. Carnegie Inst. of Wash., Contributions to Embryology, No. 21, pp. 57–85 (pp. 52, 102).

VAN DER STRICHT, R. 1911. Vitellogénèse dans l'ovule de Chatte. Arch. de Biol., t. 26, pp. 365–481 (p. 84).

VAN DURME, M., 1913. Du rôle des mitochondries dans la genèse de l'ovoplasme. Ann. et Bull. Soc. Méd., Gand, n. s., t. 4, pp. 270–278.

———— 1914. Nouvelles recherches sur la vitellogénèse des œufs d'oiseaux, etc. Arch. de Biol., t. 29, pp. 71–200 (pp. 56, 123).

VERATTI, E., 1909. Sulla fine struttura delle cellule di alcuni tumori. Boll. Soc. Med. Chir. di Pavia, vol. 23, pp. 34–45 (pp. 63, 139).

VIGUIER, G., and A. WEBER, 1912. Les formations chromidiales et mitochondriales de l'Hæmogregarina sergentium Nicolle, chez le Gongylus ocellatus. C. rend. Soc. Biol., t. 73, pp. 92–93 (p. 53).

VOINOV, D. N., 1903. La spermatogénèse d'été chez le Cybister roeselii. Arch. de Zool. exp. et gén., pp. 173–260 (p. 55).

VONWILLER, P., 1915. Die Sphäroplasten von Amœba proteus. Anat. Anz., Bd. 48, pp. 485–488 (p. 53).

WARBURG, O., 1910. Oxydationen in lebenden Zellen nach Versuchen am Seeigelei. Hoppe-Seylers Zeitschr. f. physiol. Chem., Bd. 66, pp. 305–340 (p. 147).

WELCH, W. H., 1888. Cartwright lectures on the general pathology of fever. Medical News, vol. 52, pp. 393–405 (p. 139).

WEYGANDT, C., 1907. Beiträge zur Kenntnis der Spermatogenese bei Plagiostoma girardi. Zeitschr. f. wiss. Zool., Bd. 88, pp. 249–290 (p. 54).

WILDMAN, E. E., 1913. The spermatogenesis of Ascaris megalocephala with special reference to the two cytoplasmic inclusions, the refractive body and the mitochondria: their origin, nature and rôle in fertilization. Jour. Morph., vol. 24, pp. 421–450 (pp. 45, 49).

WILKE, G., 1907. Die Spermatogenese von Hydrometra lacustris L. Jena. Zeitschr. f. Naturw., Bd. 42, pp. 669–720 (p. 55).

WILSON, E. B., 1914. The bearing of cytological research on heredity. Proc. Roy. Soc., Series B, vol. 88, pp. 333–352 (p. 96).

———— 1916. The distribution of the chondriosomes to the spermatozoa in scorpions. Science, n. s., vol. 53, pp. 539 (pp. 55, 94).

WOODLAND, W. N. F., 1911. On some experimental tests of recent views concerning the physiology of gas production in teleostean fishes. Anat. Anz., Bd. 40, pp. 225–242 (p. 147).

ZIMMERMANN, A., 1893. Sammel-Referate aus dem Gesammtgebiete der Zellenlehre. Bot. Centralbl., Hefte 3, p. 215 (pp. 46, 57).

ZOJA, F. and R., 1891. Intorno ai plastiduli fucsinofili (bioblastuli dell' Altmann). Mem. Ist. Sc. Milano. Tomo 16, pp. 237–270 (pp. 42, 51, 53–56).

THE DEVELOPMENT AND REDUCTION OF THE TAIL AND OF THE CAUDAL END OF THE SPINAL CORD.

By Kanae Kunitomo,

Nagasaki Medical School, Nagasaki, Japan.

Four plates, two text-figures

CONTENTS.

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 Physiologicæ, 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 Verlangerung.* Ecker (1851–59) referred to it as *Schwanzformige Korperende,* 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 (1880a), 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 tuberele; 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 Brugsch (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 Schwanz-
höcker in den Steisshöcker um, so wird durch den Zug der Haut, deren Wachstumrichtung
der der Steisswirbel entgegengesetzt ist, der Schwanzfaden von der Achse der Kaudalwirbel
entfernt und mit der Haut aufwärts mitgenommen (cfr. Embryo Dü. 4½ cm.). 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 Bindege-
webszügen mit der kaudalen Fläche des letzen 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 'vestiges coccygiens' von Tourneux und Hermann (s. o.) oder 'kaudalen Rücken-
marksreste' 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 per-
sisting 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 *vestiges 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. CR 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., ×70; embryos 17 to 38 mm., ×50; embryos 39 to 52 mm., ×30; embryos 67 to 125 mm., ×20.

Table of Embryos Studied.

Length (CR) of embryos	Cata-logue No.	No. of somites.	No. of spinal ganglia.	Segmental level of eloaca.	Extension of caudal end of spinal eord.	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.........	.....Do..........	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 Froriep 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 X 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 vertebræ 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 I. 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 X 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 vertebræ, and constitutes the primordium of the caudal ligament. As is well known, the sclerotomes of the somites develop into not only primitive vertebræ, 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 vertebræ. 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 vertebræ, 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 vertebræ, 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 thoughout. 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 vertebræ, the last consisting of precartilage 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 vertebræ. 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 vertebræ. 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 vertebræ, and the dorsal portion of the thirty-third, have become converted into cartilage, whereas the remainder of these two vertebræ still consists of precartilage 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 vertebræ 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 vertebræ. The last is small and contains the winding part of the chorda dorsalis. The spinal cord narrows between the thirty-first and thirty-second vertebræ, 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 vertebræ 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 vertebræ, 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 vertebræ, and at thirty-first and thirty-second vertebræ the column presents a typical curve. The chorda dorsalis shows a spindle-shaped swelling between the vertebræ, 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 vertebræ, 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 vertebræ and 31 spinal ganglia. The vertebral column curves ventrally at the thirty-first and thirty-second vertebræ. 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 vertebræ. 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 vertebræ, 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 vertebræ 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 sadral 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 vertebræ. 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 vertebræ 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 vertebræ, 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 vertebræ, 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 vertebræ, as shown in figure 45. The vertebral

column is bent ventrally between the thirtieth and thirty-first vertebræ, forming an obtuse angle and creating the typical coccygeal curve. Within the bodies of the vertebræ, 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 vertebræ 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 vertebræ. 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 vertebræ, 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 vertebræ, 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, *res. m. co.*) is situated dorso-caudal to the thirty-third and thirty-fourth vertebræ. 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 vertebræ, 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 vertebræ 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 vertebræ 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 vertebræ 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 vertebræ, contains no lumen, and the epithelial cells are undergoing degeneration. The other is situated dorsally between the thirty-third and thirty-fourth vertebræ 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 vertebræ, and the dura mater becomes adherent to the conus medullaris between the twenty-eighth and twenty-ninth vertebræ, 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 vertebræ 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 humain), 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 vertebræ. 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 vertebræ, 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 vertebræ, 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 vertebræ can be recognized at the caudal end of the vertebral column: (1) the vertebræ which have developed from the sclerotomes into precartilaginous or primitive vertebræ; (2) the incomplete primitive vertebræ, or the parts of the thirty-sixth and thirty-seventh sclerotomes which form the last vertebræ; (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 vertebræ, 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 vertebræ 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 vertebræ 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 vertebræ. 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-vertebræ 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 vertebræ. 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 vertebræ 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 vertebræ 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 vertebræ. The lost-vertebræ 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 vertebræ; (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 vertebræ 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 vertebræ 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 vertebræ. In the 25 and 27 mm. embryos the lost-vertebræ 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-vertebræ 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'integument 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 Brugsch, 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 thoughout 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 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

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.

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 *restiges 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 refléché. 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 vertebræ. 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 vertebræ. 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 vertebræ, 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 vertebræ 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 vertebræ, 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-vertebræ 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 vertebræ, 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
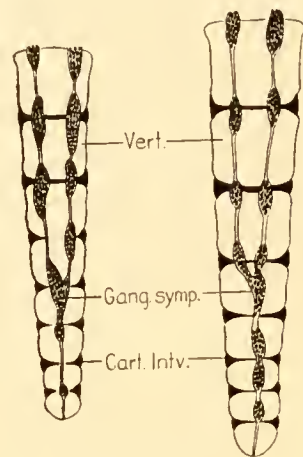
misnomer. Sometimes it appears as a rounded eminence and was therefore termed caudal tubercle by Unger and Brugsch.

(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 vertebræ; (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 vertebræ.

(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 vertebræ.

(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.

BIBLIOGRAPHY.

ARGUTINSKY, P. Ueber die Gestalt und die Enstehungsweise des Ventriculus terminalis und über das Filum terminale des Rückenmarkes bei Neugeborenen. Arch. f. mikrosk. Anat., 1898, LII, 501–530.

BARDEEN, C. R. Studies of the development of the human skeleton. The development of the lumbar, sacral and coccygeal vertebræ. Amer. Jour. Anat., 1905, IV, 266–302.

BARTELS, MAX. Die geschwanzten Menschen. Arch. f. Anthropol., 1884, XV, 45–131.

BERRY, J. Baby with a tail. Memphis Medical Journal, 1894, XIV, p. 105.

BRAUN, M. Entwickelungsvorgänge am Schwanzende bei einigen Säugethieren mit Berücksichtigung der Verhältnisse beim Menschen. Arch. f. Anat. u. Physiol. (Anat. Abth.), 1882, 207–241.

BRUGSCH, T. Zur Frage der Schwanzbildung beim Menschen. Zeitschr. f. Heilk., 1907, XXVIII (Abth. f. path.), 155–161.

—— and E. UNGER. Die Entwicklung des Ventriculus terminalis beim Menschen. Arch. f. mikrosk. Anat., 1903, LXI, 220–232.

CLARKE, J. L. Further researches in the gray substance of the spinal cord. Philosoph. Trans. Royal Soc. London, 1859, CXLIX.

DARWIN, C. R. The Descent of Man. 1871, I.

DICKINSON, A. A child with a tail. Brooklyn Medical Journal, 1894, VIII, p. 568.

ECKER, A. Icones Physiologicæ. Erläuterungsgeschichte. Leipzig, L. Voss, 1851–59.

——. Der Steisshaarwirbel, die Steissbeinglaze, und das Steissbeingrübschen wahrscheinliche Ueberbleibsel embryonaler Formen in der Steissbeingegend beim ungeborenen und der Wachsenen Menschen. Arch. f. Anthropol., 1880, XII, 129.

——. Beiträge zur Kenntniss der äusseren Formen jünster menschlicher Embryonen. Arch. f. Anat. u. Physiol. (Anat. Abth.), 1880, 403–406.

——. Besitzt der menschliche Embryo einen Schwanz? Arch. f. Anat. u. Physiol. (Anat. Abth.), 1880, 421.

FOL, H. Sur la queue de l'embryon humain. Comptes rend. 1885, Janvier-Juin, 1469–1472.

GERLACH, L. Ein Fall von Schwanzbildung bei einem menschlichen Embryo. Morph. Jahrb., 1880, VI, 106–124.

HARRISON, R. G. On the occurrence of tails in man, with description of the case reported by Dr. Watson. Johns Hopkins Hosp. Bull., 1901, XII, p. 96.

HIS, WILHELM. (a) Anatomie menschlicher Embryonen. Leipzig, 1880, I, p. 89.

——. (b) Ueber den Schwanzthiel des menschlichen Embryo. Arch. f. Anat. u. Physiol. (Anat. Abth.), 1880, 431–440.

KEIBEL, FRANZ. Ueber den Schwanz des menschlichen Embryo. Arch. f. Anat. u. Physiol. (Anat. Abth.), 1891, 356–388.

KEIBEL, F., and F. P. MALL. Human embryology. J. B. Lippincott, Philadelphia, 1910.

KOHLBRÜGGE, J. H. F. Schwanzbildung und Steissdrüse des Menschen und das Gesetz der Ruchschlagsvererbung. Naturk. Tijdschr. Nederl. Indië. Batav., 1898, LVII, 163–195.

v. KÖLLIKER, A. Gundriss der Entwicklungsgeschicht des Menschen und der höheren Thiere. 2d ed., 1884. Leipzig, Engelmann.

KONSTANTIOWITSCH, W. Zur Frage der Schwanzbildung beim Menschen. Zeitschr. f. Heilk. path. Anat., 1907, XXVIII.

KRAUSE, W. Der Ventriculus terminalis des Rückenmarks. Arch. f. mikrosk. Anat., 1875, XI, 216.

PYATNITSKI, IVAN I. On the question of the formation of a tail in man, and of human tails in general, according to data from the literature and personal researches. A. Muchnik, St. Petersburg, 1892, pp. 96.

RODENÄCKER, GEORG. Ueber den Säugetierschwanz mit besonderer Berüchsichtigung der caudalen Anhänge des Menschen. Freiburg, 1898, pp. 39.

ROSENBERG, E. Ueber die Entwickelung der Wirbelsäule und des Centrale carpi des Menschen. Morph. Jahrb., 1876, I, 81–198.

——. Ueber eine primitive Form der wirbelsäule des Menschen. Morph. Jahrb., 1899, XXVII, 118.

SCHAEFFER, OSKAR. Beitrag zur Aetiologie der Schwanzbildungen beim Menschen. Arch. f. Anthropol., 1892, XX, 189.

SCHWARZ. Beitrag zur Kenntniss des geschwanzten Menschen. München med. Wochenschr., 1912, LIX, 928.

TOURNEUX, F., and G. HERMANN. Sur la persistance de vestiges médullaires coccygiens pendant toute la période foetale chez l'homme et sur le rôle de ces vestiges dans la production des tumeurs sacrococcygiennes congénitales. Jour. de l'Anat. et de la Physiol., 1887, XXIII, 498–529.

TOURNEUX, F. Sur la structure et sur le développement du fil terminal de la moëlle chez l'homme. C. R. hebd. de la Soc. de Biol., ser. 9, T. 4, 1892, 340–343.

UNGER, E., and T. BRUGSCH. Zur Kenntniss der fovea und fistula sacrococcygea caudalis und der Entwicklung des ligamentum caudale beim Menschen. Arch. f. mikrosk. Anat., 1903, LXI, 151–219.

VIRCHOW, R. Schwanzbildung beim Menschen. Berlin klin. Wochenschr., 1884, XXI, p. 8.

WALDEYER, W. Die Caudalanhänge des Menschen. Sitz.-bericht. Akad. Wiss., Berlin, 1896, p. 775.

WATSON, W. T. The exhibition of a three-months infant with a caudal appendage. Johns Hopkins Hosp. Bull., 1900, XI.

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:

A. s. m. Arteria sacralis media.
Append. Caudal extension of the terminal ventricle.
Caud. non-v. Part of tail containing no vertebræ.
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.

Pia. Pia mater.
Pl. vasc. Plexus vasculosus.
Re. epend. Remnant mass of ependymal cells.
Rect. 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.

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. 584a, 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.
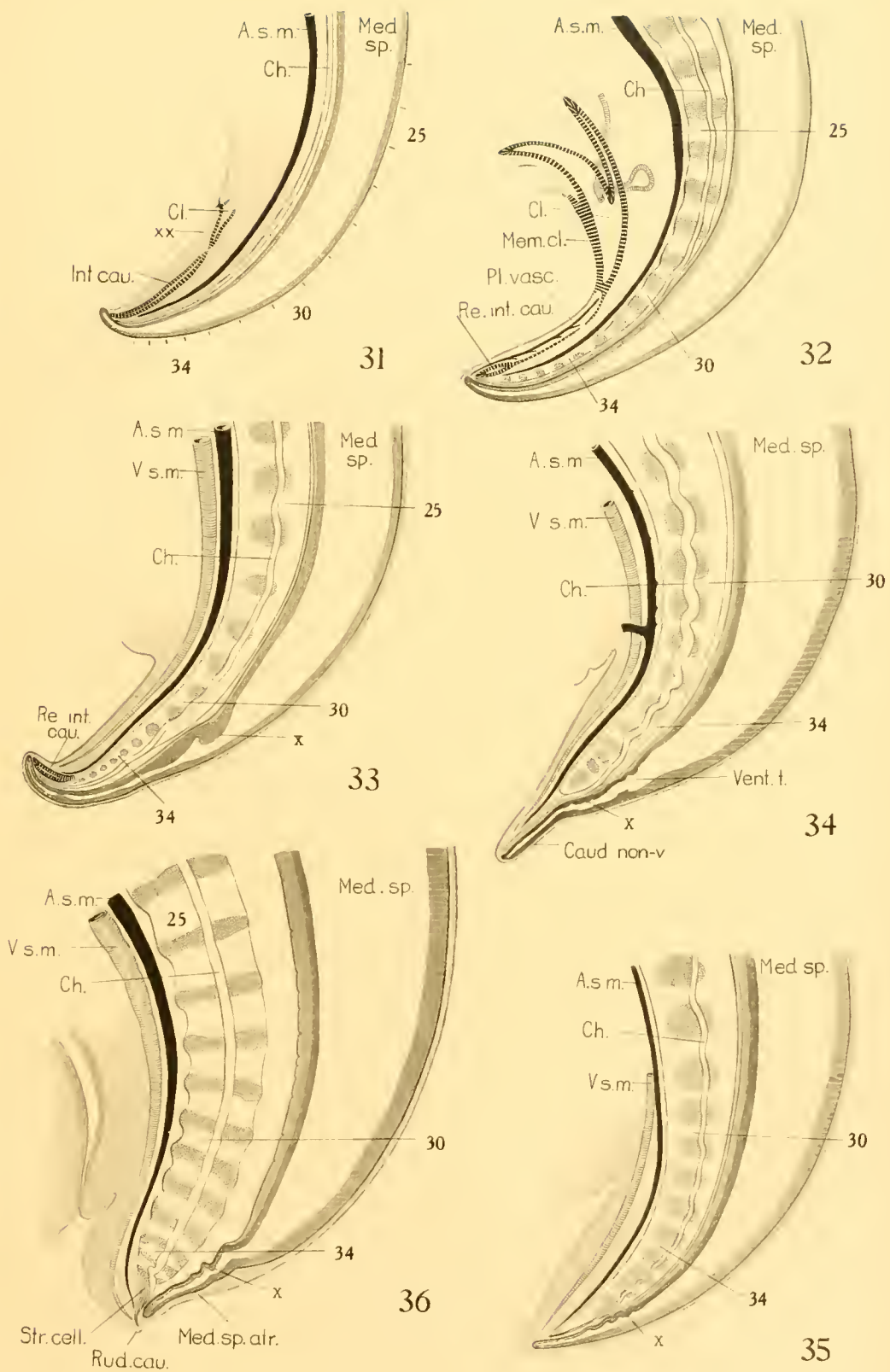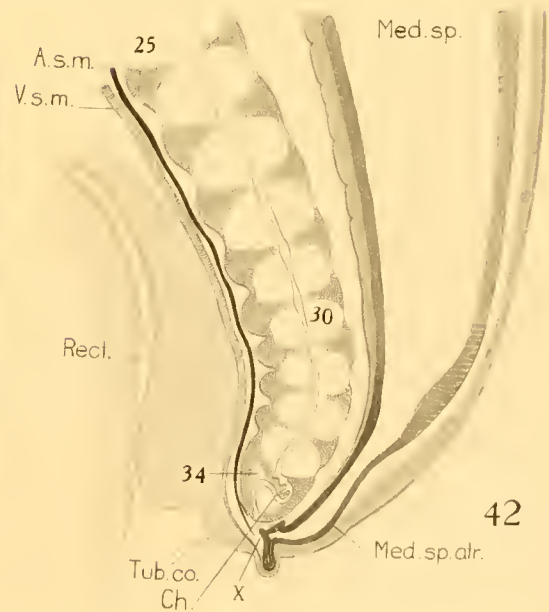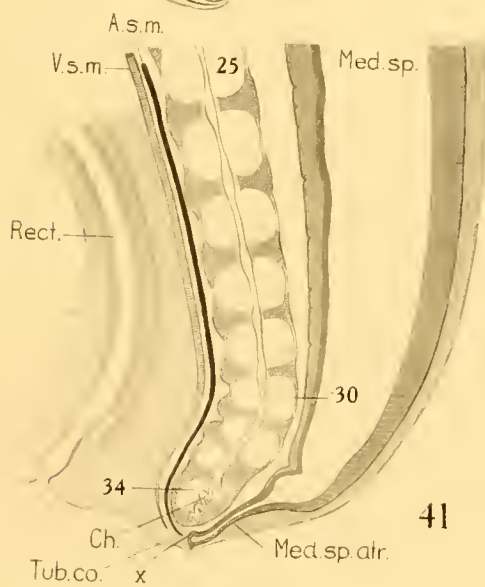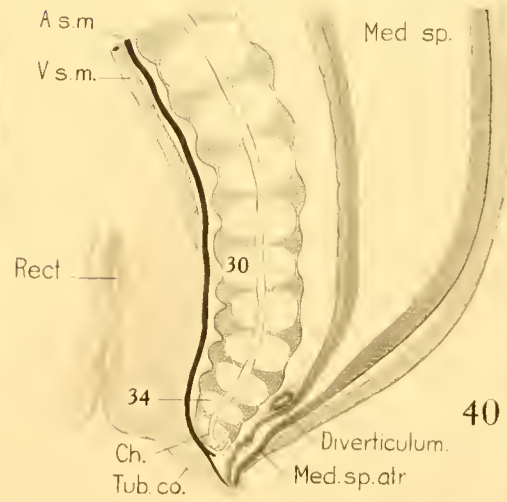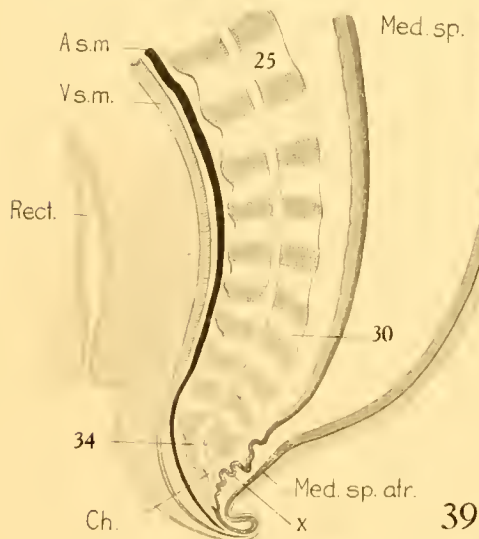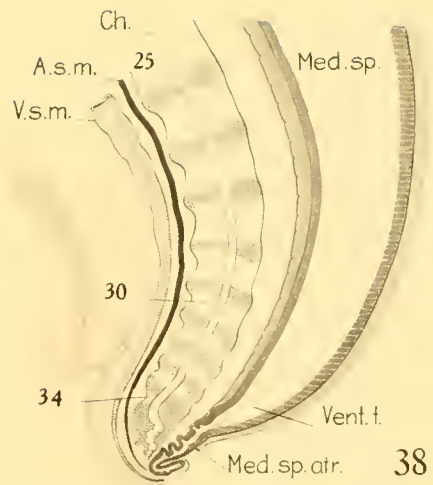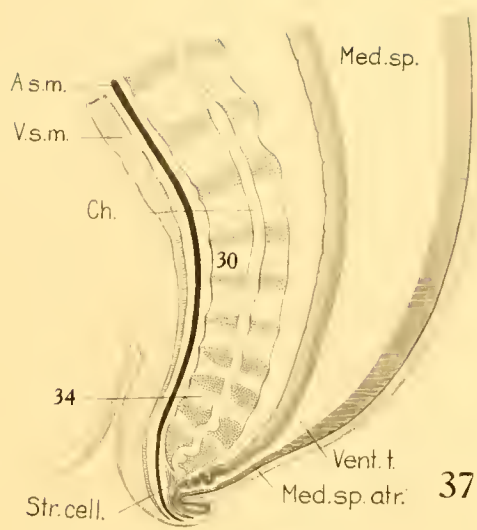
PLATE 4.

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.
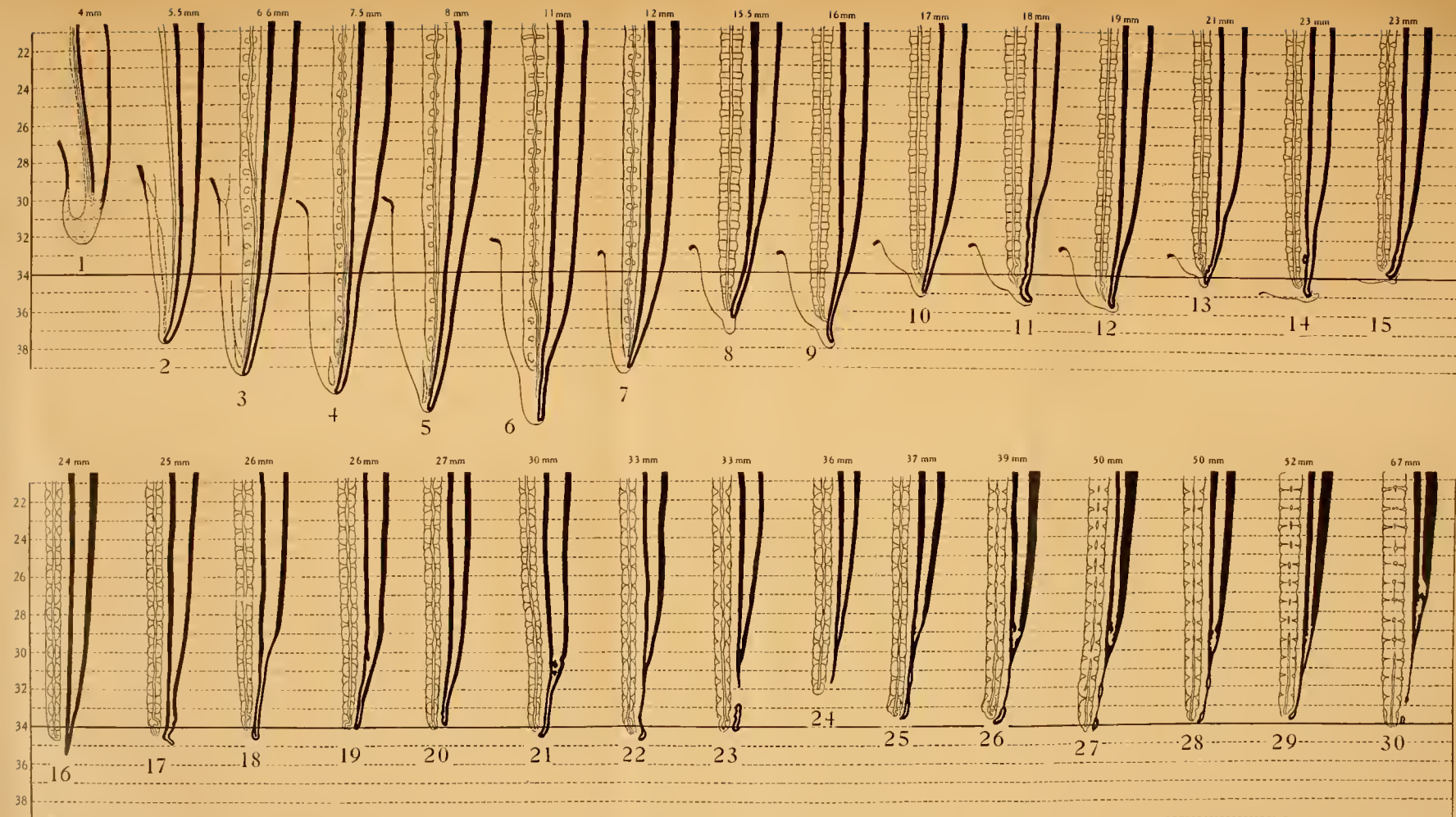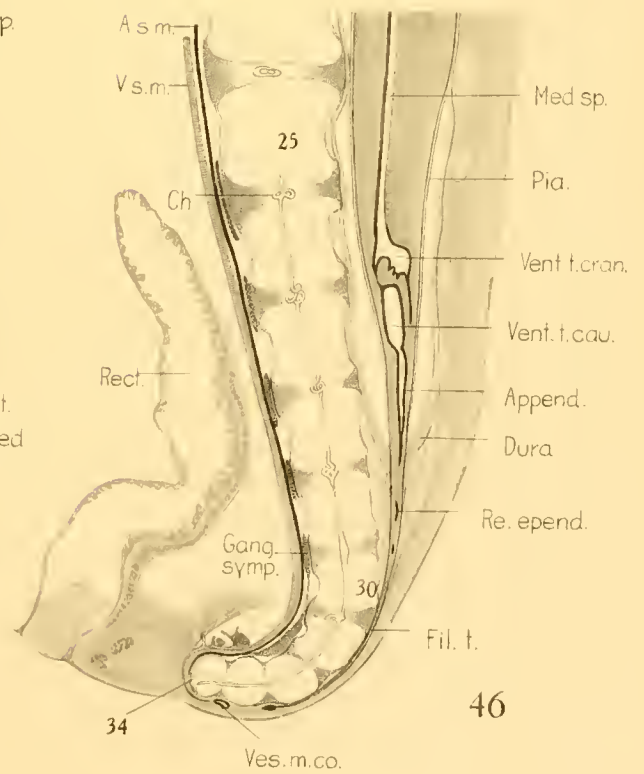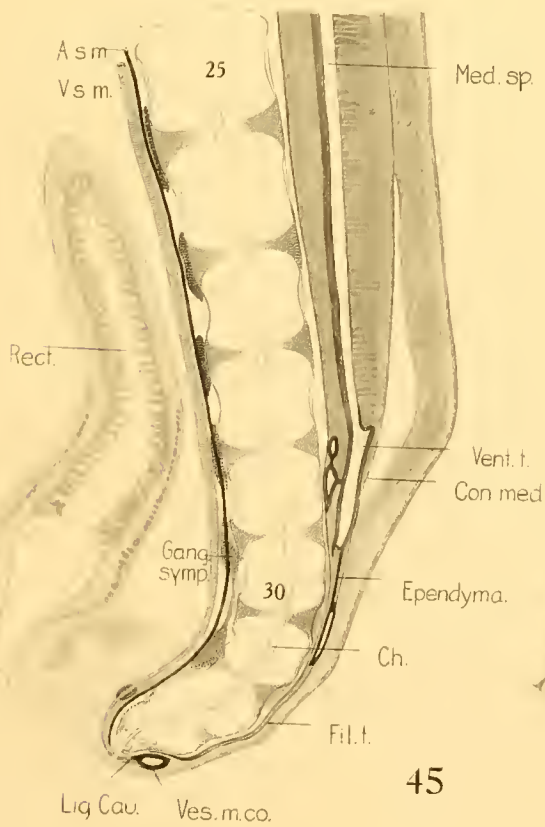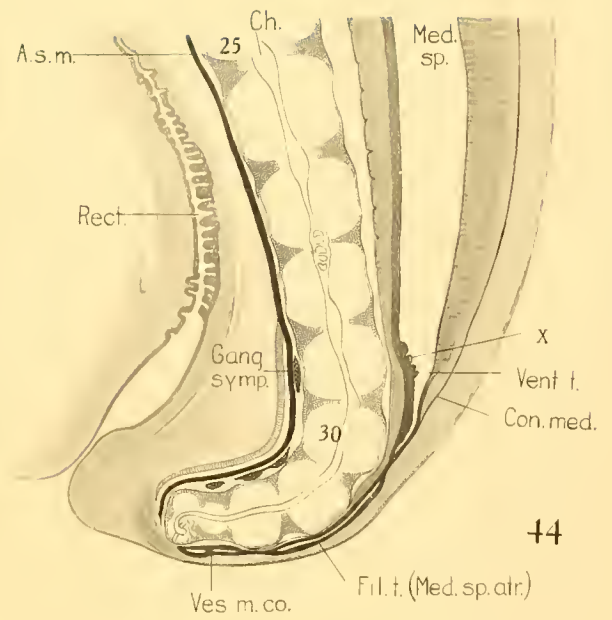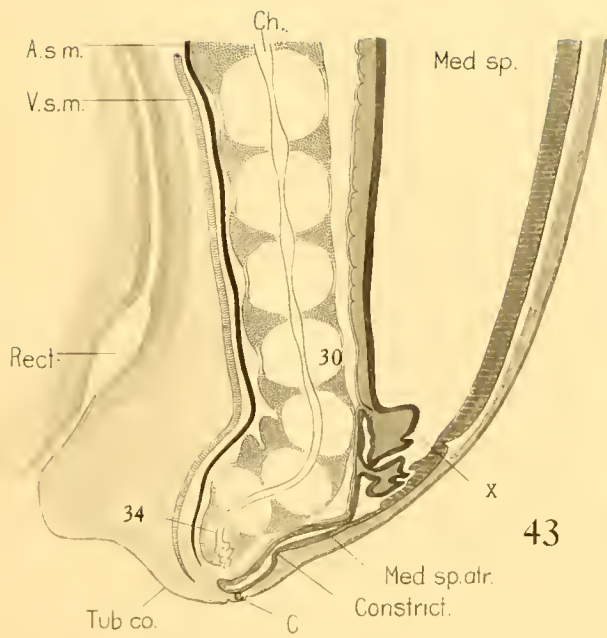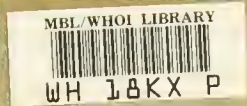
The figures on Plate I are designed to show diagrammatically the relations of the caudal end of the spinal cord and the vertebral column in a series of human embryos varying from 4 mm. to 67 mm. long, and are so arranged that the segmental levels, as indicated at the left, correspond throughout, the 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 vertebræ. 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	972
6	544	16	692	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

PLATE 2

PLATE 3

PLATE 4

43

44

45

46