A C T A   T H E R I O L O G I C A

VOL. XIII, 13: 219—260.   BIAŁOWIEŻA   20.V.1968

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Embryogenesis of the Auditory Part of the Inner Ear in the Guinea Pig

[With 1 Table, 5 Figs. & Plate IV—IX]

The process of morphological differentiation of the inner ear in the guinea pig is accompanied by characteristic histochemical transformations within the cells and tissues. The start of the functioning of the inner ear is preceded by increase in the activity of respiratory enzymes in the sensory cells and in the cells of *stria vascularis* and the appearance of glycogen grains in the hair cells. Nissl's bodies appear in the spiral cells. The inner ear is capable of receiving sounds as soon as Corti's organ is completely formed on all the turns. The youngest embryos of the guinea pig in which reaction to sounds was found in the form of microphonic potential were 80 mm long. The value of the microphonic potential increases with increasing length of the embryo and before birth attains several hundred μV. To a certain extent the curve representing increase in activity of the histochemical transformations runs parallel to the curve of increase in mean values of the microphonic potential with the growth of the embryos. During embryogenesis the different kinds of cells of the cochlear duct and spiral cells possess a Golgi apparatus of characteristic shape and location. In the spiral ganglion cells the situation of the Golgi apparatus is polar, but as from the time the embryos reach the length of 65 mm it adopts an apolar position.

The development of the auditory part of the inner ear in the guinea pig may, on the basis of the material elaborated, be divided into two periods: Period I — in which morphological and histochemical differentiations occurs. Period II — is characterised by the start of the bioelectric function of the organ of Corti.

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I. INTRODUCTION

The development of the inner ear and its adaptation to receiving sounds is a very complicated process from both the morpological and biochemical aspects. A more accurate knowledge of this development may facilitate studies on the pathogenesis of congenital defects of hearing (Töndury, 1952; Weibel, 1957).

The cytological and histochemical pictures of the elements of the inner ear only indirectly express the functional state of morphological elements and their adaptation to receiving sounds. An objective manifestation of the function of the organ of hearing during perception of sounds are electric currents (microphonic potential, summating potential, potential of auditory nerve).

Many authors have in recent years examined the question of the possibility of reception of sounds by the developing organ of hearing. Studies of this kind in humans are based on observations of the heart function of the embryo, encephalographic and clinical examinations and are also treated as an attempt at early discovery of cases of deafness (Dwornicka, 1963; Brown, 1964; Elliot, 1964; Johansson, 1964). Research on animals is concerned chiefly with the correlations between the morphological picture of the differentiating Corti's organ and manifestations of its function. Investigations are usually carried out on those species of animals which are born deaf and exhibit the final formation of the organ of hearing during the first weeks after birth.

The aim of this study is to obtain a better knowledge of the morphogenesis and development of the function of the inner ear in the guinea pig during embryonic life.

II. REVIEW OF REFERENCES

Among the basic studies dealing with development of the inner ear are those devoted to morphogenesis of the labyrinth. Streeter (1906, 1917, 1918), using models, presented the development of the osseous and membraneous labyrinth, the auditory nerve and endolabyrinthal space in man. Anson (1934), on the basis of material taken from different animals, discusses the early stages of formation of the labyrinth.

An exact description of differentiation of the structures of the Corti organ and tectorial membrane in animals was given by Van der Stricht (1918, 1920). Hardesty (1915) discusses the formation and structure of the tectorial membrane in pig embryos. Held (1926) and Kolmer (1927) in studies of a monographic character describe the formation of the organ of hearing, with particular reference to man. Altmann (1950) on the basis of extensive literature discusses the development of the organ of hearing and the mechanics of this development. Weibel (1957) carried out detailed studies on differentiation of the cochlear duct in mice. Grisanti (1957) investigated the development of *stria vascularis* in the rabbit. Vinnikov (1959, 1961) discussed the phylogenetic and ontogenetic development of the human organ of hearing.

Among other interesting studies on the embryogenesis of the inner ear we must include investigations of the development of transplantations of the labyrinth under different experimental conditions. For instance Kaan (1917) defined the capacity for transformation of an otic vesicle transplanted to the labyrinth in *Amblystoma punctatum*. Waterman (1917) observed fuller histological differentiation of the labyrinth in comparison with its anatomic development in transplanted rabbit

embryos. Kogan (1950) carried out studies on the development of the membranous labyrinth in birds under conditions of artificial tissue cultures. Similar investigations were made by Lawrance & Merchant (1953) on the otocyst of fowl and rat embryos and by Friedmann (1956; 1959; 1961) on the embryos of birds.

The histochemical observations made in recent years have contributed to a better knowledge of the development of the organ of hearing. Bélanger (1956), for instance, observed the occurrence of polysaccharides and basophil substance during the development of epithelium and connective tissue structures of the cochlea in rats. Rossi (1963) made studies of the activity of acetylocholinesterase during the embryogenesis of the spiral ganglion in the guinea pig. Titova (1965) carried out histochemical examination of the differentiation process in the inner ear in goat, rabbit and cat embryos beginning with the second half of pregnancy, and ending with animals from 10—14 days after birth. This author considers that nuclein acids play the most important part in the differentiation process of the structures of the cochlear duct.

Observations made by means of electronic microscope must be included in research work carried out recently. Friedmann (1959, 1961) discusses the ultrastructural organisation of sensory epithelium, supporting cells and intercellular space in the fowl embryo otocyst. Titova (1965) carried out observations of the development of the organ of hearing and balance in vertebrates. Kikuchi & Hilding (1965), using mice, investigated the development of the organ of hearing by means of a light and electronic microscope. These authors consider that efferent nerve endings play an important part in the development of Corti's organ. In 1966 the same authors reached the conclusion, during their examination of *stria vascularis* in mice, that the morphological structure of the cells of this stria makes it possible for them to participate in fluid transport even during the development of the ear — this also applies to the cells of the spiral prominence.

Ruben (1967) using marked tritiated thymidyne, defined the time of differentiation of the different parts of the inner ear in mice. In his opinion the cochlear duct in the region of the apical turn undergoes earlier differentiation in comparison with the basilar turn. The spiral ganglion, on the other hand, differentiates first on the level of the basilar turn, and then on the level of the apical turn.

Other studies include, in addition to the process of morphological formation of the inner ear, the development of its reactions to sounds in the form of electric currents. Studies of this kind were made on the opossum (*Didelphis virginiana*) Mc Crady, Larsell, Wever, Bray (1934—1945). Histological investigations and measurements of the microphonic potential were made by these authors on animals, beginning with the first hours after birth up to their attainment of complete maturity, and demonstrated that the morphological and functional development of the organ of hearing follow a parallel course. The opossum provides particularly convenient material for investigations of the embryogenesis of the ear as these animals are born deaf, and development of the organ of hearing follows after birth when they are living in the mother's pouch.

Schmidt & Fernandez (1963) carried out their histological investigations of the cochlear duct during the development period in the opossum and simultaneously ascertained its endocochlear potential.

Alford & Ruben (1963) defined the connection between the occurrence of Preyer's reflex, cochlear microphonic potential, potential of the auditory nerve and the histological picture of the developing cochlea in white mice, which are still deaf immediately after birth.

Kljavina & Maruseva (1963) registered the potential of the auditory nerve in cats from 2 to 30 days old. Mazo (1955) defined the microphonic potentials in rook nestlings, fowl chicks and newborn rabbits. These measurements were concerned with the bioelectric response of the inner ear to sounds of different frequencies and define the excitability threshold at these frequencies.

Änggard (1965) carried out electrophysiological investigations on the development of cochlear function in the rabbit, defined the microphonic potential, endocochlear potential, summating potential and electric response of second order auditory neurons. Animals up to 37 days old were used for those investigations.

Chodynicki (1966) described in successive studies the occurrence of enzymes, glycogen, lipides and the Golgi apparatus in the inner ear of the guinea pig during the period of embryonic life and investigated the microphonic potential recorded in embryos from 80 mm in length onwards. Chodynicki & Matwijewicz (1966, 1967, 1968) also described the relation between the microphonic potential and the state of embryonic circulation in the guinea pig.

III. MATERIAL AND STUDY METHODS

1. Histological and Histochemical Methods

The studies were made on 120 embryos of the guinea pig, obtained by Caesarean section under urethane anaesthesia. Length of embryos was measured in the crown-rump length.

Embryos up to 40 mm long were fixed in their entirety, but in the case of embryos over 40 mm in length the temporal bone was fixed after opening *bullae tympanicae* and the peri- and endolymphatic spaces of cochlea (Chodynicki, 1966).

Cooled Baker's fluid, Gendre's fluid and Aoyama's fluid were used for fixing; part of the material was placed in a thermos with dry ice. The material was embedded in paraffin and cut in series into axial sections from 3—5 μ thick. The temporal bone from embryos over 70 mm long was decalcified in 5% water solution of versene (EDTA — ethylenediaminetetraacetic acid) (Balogh, 1962). After decalcification the cochlea was embedded in paraffin or gelatine and sectioned in a plane parallel to the modiolus.

Material intended for enzymatic investigations was sectioned in a cryostat, and in the case of large embryos (over 70 mm in length) preparations were made of Corti's organ by the method of surface preparations (Vinnikov & Titova, 1959; Engström, Ades & Hawkins, 1963; Bredberg, Engström & Ades, 1965; Chodynicki, 1966).

The sections were stained with hematoxylin-eosine and by the Azan method. Reticular fibres were revealed by the Laguess and Gomori method. The McManus method was used for obtaining PAS reaction. Glycogen was stained with Best's carmine. Control preparations were subjected to the action of diastase (Bagiński, 1965). Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were discovered by means of gallocyanine and Brachet's method (Godlewski & Vorbrodt, 1954; Krygier & Godlewski, 1964). Control preparations were subjected to the action of pancreatic extract in accordance with Brachet's method (Bagiński, 1965). In addition the preparations were stained by the Himes-Moriber method (Himes & Moriber, 1956). The Golgi apparatus was discovered

by Aoyama's method. Lipids were stained with oil red O, *R* scarlet, Nile blue, osmic acid (Bielańska-Osuchowska, 1960).

Alkaline phosphatase (Al. Pase) and acid phosphatase (Ac. Pase) were examined by means of the Gomori method as modified by Godlewski & Vorbrodt, and also with the use of coupling azo dye method (Krygier & Godlewski, 1964). Succinate dehydrogenase (S.D.) was revealed by the Nachlas method, lactic dehydrogenase (L.D.) by the method used by Hess *et al.* (Gerhardt, 1961; Pearse, 1960). In addition ash pictures were made (Krygier & Godlewski, 1964).

2. Bioelectric Method (Measurements of Microphonic Potentials)

Measurements were made using 70 guinea pig embryos varying in length from 70 mm to 130 mm. Caesarean section was performed under urethane anaesthesia. The embryos were removed while maintaining blood circulation and constant temperature, and recording heart function (Chodynicki, 1966, 1967). The round window was exposed by operation on the embryos, additional anaesthesia of the embryo proving necessary in some cases. The microphonic potentials were received from the exposed round window membrane by means of a thin platinum wire electrode 10 μ in diameter insulated with collodium and introduced into the space of the middle ear under an operating microscope (Brohm, 1962; 1963).

Sounds were conveyed to the skull bones by means of an acoustic sounder connected with the screen bone vibrator of a AU type audiometer (Brohm, 1956). The end of the sounder measuring 4 mm $\times$ 4 mm was arranged on the skull bones 5 mm forwards from the margin of the open *bulla tympanica* of the left ear. Microphonic potentials were defined with frequencies of 1000, 2000 and 4000 c.p.s., with 75 db. The *CM* values were recorded on photo-sensitive paper, using a »Disa« electromyograph.

IV. MORPHOGENESIS AND HISTOCHEMISTRY OF THE DEVELOPMENT OF THE AUDITORY PART OF THE INNER EAR OF THE GUINEA PIG

1. Description of Material

Embryos 12 mm long (guinea pig no. 30, Figs. 1—3).

The auditory vesicle and the endolymphatic duct forming on the dorsal wall of the vesicle can be seen in the axial sections of embryos of this length.

The vesicle is formed from pseudostratified columnar epithelium (Figs. 1, 2). The numerous cellular nuclei of this epithelium are in various stages of karyokinesis. From the head side there is a concentration of nerve cells adjoining the wall of the vesicle (Figs. 1, 2).

Golgi apparatus occurs in the epithelium of the vesicle and endolymphatic duct as elongated formations lying in the apical part of the cells (Fig. 3). The Golgi apparatus of the nerve cells is oval in shape and in apolar position.

DNA. Preparations stained with gallocyanin reveal the presence in the nuclei of numerous large irregularly distributed chromatin granules. RNA. The cytoplasm of the pseudostratified columnar epithelium exhibits intensive pyronin absorption when the Brachet method is used. Intensive pink staining is clearly visible in the apical and basal part of the cells. These cells stain a dark blue colour with gallocyanin.

Glycogen. When the PAS method is used single small granules staining a pink colour can be seen in the cytoplasm of the cells of the epithelium. In addition mesenchymal cells lying near the epithelium stain a purple colour.

Ac. Pase (acid phosphatase) reveals slight activity in the epithelium, where it is located in the apical part of the cells. It is very active within the nerve cells. Al. Pase (alkaline phosphatase) is characterised by low activity in the whole of the epithelium.

L.D. (lactate dehydrogenase) and S.D. (succinate dehydrogenase) also exhibit only slight activity in the basal and apical parts of the epithelium cells.

Embryos 14 mm long (guinea pigs nos. 28, 35, Figs. 4, 5).

There is a tract of more intensively staining connective tissue cells round the cochlear duct, which constitute the rudiment of the cochlear capsule. Cephalad from the cochlea the vestibule (Fig. 4), utricle and endolymphatic duct can be seen.

The cochlear duct forms one and a half turns. The lumen of the duct is narrow. The surface of the cells of the pericentral part of the lower wall of the duct is covered by a thin membrane. Ganglion cells, the fibres of which are directed towards the epithelium cells, are situated at the base of turn I and II. The cochlear duct is surrounded by delicate single reticular fibres (Fig. 5).

The Golgi apparatus in the epithelium cells of the cochlear duct is similar in embryos 12 mm long. The behaviour of RNA and DNA is similar to that in preparations of embryos 12 mm long.

Only single granules can be seen in the epithelium of preparations stained by the PAS method. Numerous PAS-positive granules are situated within the forming cochlear capsule. The picture is similar to that seen when preparations are stained with Best's carmine.

Embryos 17 mm long (guinea pigs nos. 29, 33, Figs. 6, 7).

In the axial section four sections of the cochlear duct can be seen through the organ of hearing. These sections surround the nerve cells situated in the central part of the preparation.

The pseudostratified columnar epithelium of the duct is far higher on the side of the nerve cells (Fig. 6). The cochlear duct is surrounded on the outside by the labyrinthine capsule, the cells of which are transformed into hyaline cartilage. There is a small free space of the developing *bulla tympanica* in the surrounding region of the capsule from the ventral side of the embryo.

The behaviour of the Golgi apparatus, RNA and DNA is similar to that in embryos 12 and 14 mm long.

Glycogen. Preparations stained by the PAS method and Best's carmine contain pink or purple granules in the cells of the capsule. Similar granules are also present on the level of turn II in the apical part of the cells of cochlear duct wall (Fig. 7).

Embryos 20 mm long (guinea pigs nos. 9, 15, Figs. 8—11).

The cochlear duct, forming two and a half turns, surrounds the nerve fibres and the spiral ganglion cells situated in the middle. An area of lesser density of the

connective tissue cells, formed as the result of cytolysis, can be seen in the area surrounding the basal turn at the place where *scala vestibuli* and tympani form. The nuclei are observed to migrate in the direction of the base of the cells in the pseudostratified epithelium. PAS+ granules are visible in the cytoplasm of the cells of the epithelium, the cells of the connective tissue and the capsule. A clearly visible thin membrane covers the surface of the epithelium cells over the area of the basal turn.

Al. Pase — localisation of the enzyme takes place primarily in the external part of the capsule within a narrow tract of perichondrium. Al. Pase exhibits little activity in the remainder of the preparation. Ac. Pase — is located in the apical part of the epithelium cells (Fig. 9). Elongated blackened patches with fairly distinct boundaries are visible in this part of the cells. Greatest enzyme activity is encountered within the ganglion cells in the form of oval black areas situated in the marginal part of the cytoplasm (Fig. 10).

L.D. occurs in the apical part of the cells of the cochlear duct epithelium, exhibiting considerable activity in this place (Fig. 8). L.D. in the nerve cells is similarly active. S.D. exhibits very slight activity in the epithelium cells and slightly greater activity in the nerve cells.

The ash picture of the cochlea contains small, not very numerous particles which shine white against the dark background when seen under a microscope (Fig. 11).

Embryos 32 mm long (guinea pig no. 40, Figs. 12—14).

Seven sections of the cochlear duct can be seen in the axial section. The free spaces forming the *scala vestibuli* and *tympani* can be seen round the cochlear duct in the region of the basal turn. From the exterior the cochlea is surrounded by a cartilaginous capsule.

Certain differences can be seen in the epithelium of the cochlear duct, particularly in the region of the basal turn. The highest pseudostratified columnar epithelium is situated in the region of the lower wall, in the immediate vicinity of the nerve cells. Usually the lateral wall and part of the upper wall of the cochlear duct already possess simple columnar epithelium. From the pericentral side of the cochlear duct the epithelium can be observed to be slightly concave at the level of the basal turn. Mesenchymal cells are concentrated in this place, forming *limbus spiralis*. In the pericentral part of the lower wall the surface of the cells is covered by a thin *membrana tectoria* which stains pink by the PAS method.

In the medial part of the tympanic wall four cells can be distinguished with large light-coloured nuclei and intensively staining cytoplasm lying above the nuclei in the remaining cells of the lower wall — these are hair cells (Fig. 12). The cytoplasm of these cells exhibits distinct pyronin absorption, and also stains intensively with gallocyanin.

The Golgi apparatus in the cells of the lower wall can be seen as elongated formations. The hair cells and the cells of the remaining part of the cochlear duct contain Golgi apparatus in the shape of oval formations. In the nerve cells the position of the apparatus is polar (Fig. 14).

Glycogen. Very numerous grains of PAS+ can be seen in the epithelium in the region of turn IV. Far fewer grains are observed in the cytoplasm of cells on the level of the remaining turns (Fig. 13).

S.D. — the enzyme is very active in the cells of the external wall of the cochlea and in the nerve cells. L.D. — is distinguished by great activity in the cells of the cochlear duct and very great activity in the cells of the external wall of the cochlear duct and in the nerve cells.

Al. Pase — exhibits great activity within the perichondral capsule. Ac. Pase — occurs in the apical part of the epithelium and in the marginal part of spiral cells.

Embryos 35 mm long (guinea pig no. 7, Figs. 15—21).

The cochlea is surrounded by a cartilaginous capsule. The cochlear duct forms four turns, being surrounded in the region of turns I and II by the perilymphatic space. In the medial part of *scala vestibuli* and *tympanici* there are single connective tissue cells, but numerous mesenchymal cells still occur near the walls (Fig. 15).

The cochlear duct is triangular in shape in the region of the basal turn, but is oval in the area of the remaining turns. The walls of the duct are formed of morphologically differing elements (Fig. 15, 16).

Tympanic wall: *limbus spiralis* is covered by a simple columnar epithelium, while there is pseudostratified columnar epithelium in the region of the *sulcus spiralis externis.* A thin *membrana tectoria* can be seen on the surface of the epithelium of *limbus spiralis* and of the pseudostratified columnar epithelium, which extends to the outer hair cells. The outline of the inner tunnel and space of Nuel, pillars and cells of Deiters, and the inner outer hair cells lying higher, can be seen at the level of turn I in the medial part of vestibuli. The cells of Hensen adjoin the outer hair cells, then come the cylindrical cells of Claudius. *Sulcus spiralis externus* is lined with simple columnar epithelium cells.

Epithelium vestibuli lies on *membrana basilaris* containing a thin tract of fibres which stain blue with Azan. In the silvered preparations the fibres can be seen in the form of a thin bundle of black fibrils.

Reissner's membrane is formed by squamous cells, to which single connective tissue cells adhere from the side of *scala vestibuli.*

The spiral prominence is visible on the external wall. This wall is formed by the cylindrical epithelium to which the cells of connective tissue and blood vessels are adjacent. Under epithelium of *stria vascularis* and in *ligamentum spirale* cells occur possessing processes, the cytoplasms of which contain granules of brown pigment. *Ligamentum spirale* is formed of connective tissue cells and reticular fibres.

A distinct Golgi apparatus with a characteristic shape in the various cells occurs in the cells of the cochlear duct. Cells of the lower wall of the duct and Reissner's membrane possess the largest Golgi apparatus (Figs. 18—21).

RNA. The cytoplasm of the hair cells reveals the presence of fine, very abundant granular matter stained blue with gallocyanin. Similar granulation (but less abundant) occurs in the other cells of the cochlear duct.

Numerous PAS+ grains occur in the cells of *stria vascularis,* less numerous in the cells of *sulcus spiralis externus* and in the pseudostratified columnar epithelium near *limbus spiralis* (Fig. 17). A similar picture is observed after staining the preparations with Best's carmine. The hair cells do not contain PAS+ grains. The tectorial membrane stains pink by the PAS method.

Al. Pase. Very great activity of the enzyme is observed in the perichondrium. Ac. Pase is distinguished by marked activity in the apical part of cells of the pseudostratified columnar epithelium, hair cells and in the marginal part of spiral ganglion cells.

Embryos 38 mm long (guinea pig no. 34, Figs. 22—23).

The cochlear duct forms four full turns (Fig. 22). The formed perilymphatic space can be seen round the cochlear duct at the level of turns I, II and partially III. There are numerous connective tissue cells arranged in fan-shape between the external wall and the cartilaginous capsule (on the site of the forming *ligamentum spirale*). The greatest morphological differences are exhibited by the cochlear duct in the region of the basal turn. Reissner's membrane is formed from the layer of squamous cells. Single connective tissue cells, some of which exhibit atrophic features, lie outside this layer.

Tympanic wall: *limbus spiralis* covered with simple cuboidal epithelium can be seen near the centre. Pseudostratified columnar epithelium covered with a thin tectorial membrane is located on *membrana basilaris* on the site of the future *sulcus spiralis internus*. Supporting cells, hair cells, inner tunnel of Corti and space of Nuel are clearly visible.

Sulcus spiralis externus is formed of simple columnar epithelium. In preparations stained by the Azan method fibres staining blue and purple by the PAS method can be seen within *membrana basilaris*. The tracts of fibres within *membrana basilaris* can be seen in the silvered preparations. These fibres surround *sulcus spiralis* on one side but pericentrally, in the connective tissue of *limbus spiralis*, are arranged in a fan-shape (Fig. 23). The external wall, divided by *prominentia spiralis*, is formed of cuboidal cells and the connective tissue cells closely adhering to it.

Preparations stained with gallocyanin exhibit intensive staining of cytoplasm of the inner ear cells. Within the nuclei numerous fairly large chromatin particles can be seen.

Glycogen. Preparations stained by the PAS method and with Best's carmine reveal the presence of grains staining pink in the epithelium of turns III and IV and in the epithelium of the external wall of all the turns.

Embryos 40 mm long (guinea pig no. 6, Fig. 24).

The cochlear duct forms four turns and is triangular in shape at the level of turns I and II, and oval on the remaining two. The cochlear duct is surrounded by the perilymphatic space in the area of the basal and second turn. The epithelium of this duct is morphologically differentiated over the areas of turns I and II and partly III.

Glycogen. In preparations stained by PAS method granules stained pink can be seen in the cells of *stria vascularis* and cells of the wall of the cochlear duct.

Al. Pase — some increase in the activity of the enzyme is observed in the cochlear duct epithelium, with the exception of Reissner's membrane. In addition black patches are observed in the ganglion cells and places within the cell membrane of ganglion cells (Fig. 24).

Embryos 45 mm and 50 mm long (guinea pigs no. 14, 50, Figs. 25—27).

The differentiation process continues in the cochlear duct. In the basal part of the duct, in preparations stained by the Azan method, a tract of fibres arranged in fan-shape and staining blue can be seen in *limbus spiralis*. This tract is also visible in preparations silvered by the Gomori method. Fibres lying in the basal part of the duct also stain red with the use of the PAS method (but only over the area of the basal membrane).

Limbus spiralis is covered by cuboidal cell epithelium at the level of three turns, and a similar membrane is present in *sulcus spiralis externus*, but pseudostratified epithelium occurs in *sulcus internus*. Reissner's membrane is formed of simple squamous epithelium and a layer of connective tissue cells. *Prominentia spiralis* is distinctly visible on the external wall. Blood vessels are located under the epithelium. Numerous pigment cells occur within *stria vascularis*.

RNA. The cytoplasm of cells of the lower wall of the cochlear duct, and particularly the hair cells, exhibit distinct pyronin absorption, and similar absorption is observed in cells of *limbus spiralis* and in the cells of *stria vascularis*. Single granules stained with pyronin can be seen in the cytoplasm of ganglion cells.

When staining is carried out by PAS method numerous granules can be seen in the cells of *stria vascularis*, in the cells of *sulcus spiralis* in its pericentral part near *limbus spiralis* and in the region of the inner tunnel of Corti. In addition *membrana basilaris* and *membrana tectoria* stain purple. Outstandingly strong PAS+ reaction is observed in the cartilage and nerve cells.

L.D. exhibits considerable activity in cochlear duct epithelium, chiefly in the apical part of cells of the external wall and hair cells (Fig. 26). S.D. exhibits considerable activity in nerve cells of *ganglion spirale*.

Al. Pase. Great activity is observed in the cartilaginous capsule. Moderate activity can be seen in cells of the forming organ of Corti, and slightly greater activity in *stria vascularis* and Reissner's membrane (Fig. 25). Ac. Pase. is most active in the nerve cells of *ganglion spirale*, and is also located in the apical part of cells of *papilla basilaris*, when it can be seen in the form of lengthways black patches.

The ash picture contains fairly numerous small particles in cells of the cochlear duct epithelium and in connective tissue (Fig. 27). When seen against a dark background these particles give a white glow, and singly — a blue or pink glow.

Embryos 55 mm long (guinea pigs nos. 16, 17, Figs. 28—30).

The cochlear duct consists of four full turns, and is surrounded by perilymphatic space. Mesenchymal cells are present in the marginal part of these spaces. The duct is triangular at the level of all turns. Hair cells, supporting cells, pillars, and cells of Deiters and Hensen can be seen within the lower wall. *Limbus spiralis* is covered with simple columnar epithelium. *Sulcus spiralis internus* is filled with pseudostratified epithelium. The high cells of this epithelium lying near *limbus spiralis* undergo cytolytic processes. Vacuolisation in the cytoplasm is observed earliest; the cellular nuclei next undergo karyolysis, the Golgi apparatus being the last to undergo change. *Sulcus spiralis internus* is formed in this way. The vacuolised cytoplasm in preparations stained with HE (Fig. 29) is evidence of the presence of cytolysis of the cells of the pseudostratified columnar epithelium. Accumulation of PAS + granules is observed in this place (Fig. 30).

Cells of *limbus spiralis* and *sulcus spiralis* are covered by *membrana tectoria*, which extends over the area of all the turns to the outer hair cells. Reissner's membrane is formed of two layers of squamous cells.

A large oval Golgi apparatus occurs in hair cells, cells of *limbus spiralis* and in cells of *prominentia spiralis*, but in cells of *stria vascularis* the Golgi apparatus is barely visible. In the nerve cells the apparatus surrounds $1/2$ of the cellular nucleus.

Numerous RNA grains can be seen in the cells of the perichondrium.

PAS method — granules stained purple are observed in the pseudostratified columnar epithelium near *sulcus spiralis*, in the region of the forming inner tunnel

and in *stria vascularis*. *Membrana basilaris* and *membrana tectoria* also stain with the PAS method.

L.D. — exhibits very great activity in the cells of *stria vascularis*, in the hair cells and ganglion cells, but lesser activity in the remaining tissues (Fig. 28). S. D. — exhibits distinct activity only in the cells of *stria vascularis* and the hair cells.

Embryos 65 mm long (guinea pigs nos. 13, 18, 24, Figs. 31—36).

The cochlear duct exhibits continued differentiation of *sulcus spiralis internus* over the area of turns I, II and partly III (Fig. 31). In the apical part of the cells of this sulcus there is a flattened Golgi apparatus (Fig. 34). Within *zona pectinata* of *membrana basilaris* two tracts of fibres can be seen, between which the flattened cells are located. *Zona arcuata* of *membrana basilaris* contains one layer of fibres. Mesenchymal cells are located below the fibres of both tracts. There are numerous areas of ossification in the cartilaginous capsule of the cochlea, with the exception of that part of the capsule on the same level as the final turns of the cochlea. Grains of glycogen appear in the hair cells (single cells are also observed in the ganglion cells). *Limbus spiralis* is covered by *membrana tectoria* extending from the other side to the outer hair cells (Fig. 33). The position of the Golgi apparatus in the ganglion cells is now apolar (Fig. 35). The external wall of the cochlear duct is also differentiated to a great extent (Fig. 32).

RNA — the cytoplasm of the hair cells contains very fine granular matter staining with pyronin. Single Nissl's bodies appear in the cytoplasm of the ganglion cells. There are also numerous grains of RNA in the cells of the periosteum.

Lipids — small spherical formations about 6 μ in diameter staining with *R* scarlet, oil red O and blackening with osmic acid, can be seen in the cells of Hensen over the areas of turns III and IV. The myelin sheaths of the cochlear nerve fibres also stain black (Fig. 36).

Ac. Pase — the activity and localisation of the enzyme have not undergone any great changes. Al. Pase — is characterised by great activity in the cartilaginous capsule over an area corresponding to the occurrence of areas of ossification.

L.D. is very active in the epithelium of the cochlear duct, in *stria vascularis* and in the nerve cells. S.D. — is distinguished by very great activity in the cells of *stria vascularis* and in the nerve cells, and by slightly lesser in the hair cells.

Embryos 70 mm long (guinea pigs nos. 19, 32).

The cartilaginous capsule reveals the presence of numerous areas of ossification. The cochlear duct forms four turns with a formed perilymphatic space, with the exception of turn IV. *Limbus spiralis* is covered by cells, the lower limits of which are invisible. *Sulcus spiralis internus* is located in the space of two turns. On the exterior from *sulcus spiralis internus* a few pseudostratified columnar epithelium cells can be seen, also pillars, the inner tunnel, space of Nuel, outer hair cells and cells of Hensen. The layer of simple columnar epithelium is situated in *sulcus spiralis externus*.

When the PAS method is used grains stained purple occur in the cytoplasm of cells of *sulcus spiralis internus et externus* in the hair cells and in supporting cells.

The external wall is divided into two parts of *prominentia spiralis*. The upper part contains numerous blood vessels under the epithelium. PAS+ grains, also staining with Best's carmine, occur under the epithelium of *stria vascularis* and in

the spaces of *ligamentum spirale*. The cytoplasm of cells of ganglion spirale contain slightly more numerous grains of ribonucleic acid.

Embryos 75 mm long (guinea pig no. 23).

Ossification of the whole cochlear capsule has taken place. Grains staining with Best's carmine can be seen in the external and internal hair cells and partly in the supporting cells and cells of *sulcus spiralis externus*. These grains occur most numerously within the cells of turn IV.

Embryos 80 mm long (guinea pigs nos. 10, 12, 27, 31, 48).

The external and internal strata of the sheath surrounding the inner ear is by now formed of bone tissue, but areas of cartilaginous tissue are observed in the medial stratum of the sheath.

The organ of Corti is morphologically differentiated at the level of all the turns, but the inner tunnel of Corti is least distinctly formed over the area of turn IV.

There is a large oval Golgi apparatus in the hair cells.

The cytoplasm of the hair cells stains with gallocyanin a faint dark blue.

The external hair cells contain numerous PAS+ grains at the level of turns III and IV.

Al. Pase. Slight activity of the enzyme is observed at the different turns of the cochlea, the greatest over the area of turn IV. A similar picture can be seen in preparations incubated in order to reveal Ac. Pase.

Embryos 85 mm long (guinea pigs nos. 3, 22, 41, 46, 49, Figs. 37—41).

Areas of cartilaginous tissue can be seen in the medial part of the sheath. The cochlear duct is completely formed, the *sulcus spiralis internus*, inner tunnel of Corti and Nuel's space are clearly visible over the area of all turns (Fig. 37). There are single mesenchymal cells near the *membrana basilaris* in the region of *scala tympani*. *Zona pectinata* of *membrana basilaris* is formed by two strata of fibres, between which there are elongate cells.

RNA. There are numerous Nissl's bodies in the cells of the spiral ganglion (Fig. 38).

Numerous glycogen grains fill the outer hair cells of isolated turn IV, and can also be seen in the supporting cells and cells of *sulcus spiralis externus* (Fig. 39). The number of grains gradually decreases towards turn I. A similar picture can be seen after staining with Best's carmine.

Lipids (isolated turn IV) — there is a tract of spherical formations on either side of the inner tunnel of Corti, staining an orange colour with oil red O and an pink colour with Nile blue. The outer tract corresponds to cells of Hensen, the inner to cells of *sulcus spiralis internus* (Fig. 40).

Al. Pase. The enzyme occurs in the hairs of the external and internal sensory cells (Fig. 41).

Embryos 90 mm long (guinea pigs nos. 21, 37, 38, 39, 46, 91, Fig. 42).

The capsule surrounding the cochlea contains areas of hyaline cartilage in the medial part. Morphologically the cochlear duct is similar in appearance to a picture

of the inner ear in adult animals. The clearly visible *prominentia spiralis* on the outer wall is remarkable — below prominentia there is the space of the spiral ligament.

Fibres of *membrana basilaris* stain with the Azan method, PAS and Gomori's method (Fig. 42).

Glycogen — numerous grains are visible in the hair cells, single grains in the supporting cells.

Lipids — osmic acid — small oval formations staining black with osmic acid are situated on the external side of the organ of Corti. A similar picture can be seen in preparations stained with *R* scarlet.

L. D. — very numerous dark blue grains are observed in the hair cells, particularly in the region of turn IV, slightly less numerous but completely filling the cytoplasm, in the sensory cells of the basal turn. In the region of turn IV, lactic dehydrogenase is also visible in the endings and nerve fibres near the internal hair cells. S. D. is also characterised by considerable activity in the hair cells.

Embryos 100 mm long (guinea pigs nos. 47, 64, 65, 93, Figs. 43—45).

Ac. Pase is very active in cells of *stria vascularis*, in cells of Claudius and in the supranuclear space of the hair cells (Fig. 45). Al. Pase occurs chiefly in cells of *stria vascularis*. The cytoplasm of the ganglion cells contains very numerous Nissl's bodies.

L. D. the enzyme is very active in the external hair cells; formazan grains give the cells a dark blue colour, which is almost black in places. The activity of the enzyme is slightly less in the internal hair cells (Fig. 43). In addition formazan grains are present in the nerve cells and fibres and in the nerve endings (Fig. 44) and in the cells of *stria vascularis*. This applies to turns IV and III, while in the region of turns I and II the formazan grains are not so numerous. S. D. is very active in the internal and external hair cells and in the cells of *stria vascularis*.

Embryos 110 mm long (guinea pigs nos. 64, 71, 92).

The basal parts of the hair cells stain an intense purple colour, with PAS method. Al. Pase — is distinguished by great activity in the hair cells and particularly great activity in *stria vascularis*. Ac. Pase — the enzyme can be seen in the hair cells, and in addition within the cells of *stria vascularis*. S. D. and L. D. are most active in the hair cells and cells of *stria vascularis*.

Embryos 120 mm long (guinea pigs nos. 1, 45, Figs. 46—48).

The Corti's organ in embryos of this length is similar to the picture of this organ seen in adult animals (Fig. 46). Golgi apparatus — is oval in shape in the hair cells, and is situated in an apolar position in the ganglion cells (Fig. 48). Glycogen — The outer hair cells contain very numerous grains staining with carmine. S.D. — the enzyme is very active in the internal and external hair cells, and also occurs in cells covering *limbus spiralis* (Fig. 47). L. D. — also very active — the hair cells vary in shade from dark blue to black.

Lipids — a preparation stained with Nile blue reveals the presence of spherical pink lipid formations 6 μ in diameter, located on the outer side of the spiral organ within the cells of Hensen.

2. Discussion of Results

The epithelium of the membranous labyrinth originates from the ectoderm, while the outer wall of the membranous labyrinth and osseous labyrinth develop from mesenchyme (Streeter, 1906; Anson, 1934; Godlewski, 1948; Altman, 1950; Patten, 1948, 1963; Bochenek & Reicher, 1965). At the 6—9 somite stage a thickening forming the placode appears in the lateral convexity of the neural plate. The placode changes into the otic vesicle as the result of the sinking of the central part and the margins converging.

The further development of the auditory part of the inner ear in the guinea pig may, on the basis of the material elaborated, be divided into two periods:

Period I — in which differentiation occurs in the spiral ganglion of the cochlear duct together with formation of the organ of Corti, the perilymphatic space and the osseous labyrinth (embryos up to 80 mm long).

Period II — is characterised by the start of the bioelectric function of the organ of Corti and covers continued improvement, both morphological and bioelectric, of the organ receiving sound (embryos from 80 to 120 mm long).

2.1. First period of development — morphogenesis

At the beginning of this period, the otic vesicle develops and from its dorsal part the endolymphatic duct is then formed. The otocyst in turn divides into two parts: the upper, from which the utricle and membranous semicircular canals form, and the lower, from which the saccule and cochlea develop. The lumen of the saccule becomes convex in the part directed to the ventral side of the embryo and forms the cochlear duct (Figs. 1, 2, 3, 4).

On the exterior from the cochlear duct in embryos 14 mm long there is a stratum of intensively staining mesenchymal cells, which form the rudiment of the cochlear capsule. These cells next form into hyaline cartilage, which forms the capsule by the time the embryos are 17 mm long. In embryos 60 mm long, beginning from the basal turn, ossification begins which gradually (by the time the embryos are 75 mm long) includes the whole capsule.

The mesenchymal cells are observed to become less dense, as the result of cytolysis, in the area surrounding the cochlear duct, which during this period is still oval in the cross-section and this less dense area forms the beginning of *scala vestibuli* and *tympani*. This space, at first surrounding the cochlear duct over the area of the first turns, extends to turn IV in embryos 55 mm long (Figs. 6, 13, 15, 22).

2.1.1. Ganglion spirale of the cochlea. Nerve cells of the acousticofacial ganglion can be seen near the anterior wall of the otic vesicle (Figs. 1, 2). Numerous karyokinetic divisions are observed in these cells. During the further development of the otocyst, the nerve cells migrate to its pericentral side, where after the cells of the facial (geniculate) ganglion have separated, the upper part — vestibular ganglion and the lower part — the spiral ganglion of the cochlea — become separate. The cochlear ganglion cells, as the cochlear duct grows, are distributed along its pericentral wall (Fig. 6) and simultaneously the diameter of the cells increases. These cells are characterised by the polar position of the Golgi apparatus, which does not take up an apolar position until the embryos are 65 mm long. The first PAS + grains and Nissl's bodies appear in the cells of embryos of this length (Figs. 1—4, 6, 8—10, 14, 22, 24, 35).

2.1.2. Cochlear duct. During the first period of formation of the cochlear duct it increases in length and simultaneously adopts a spiral arrangement. The walls of the duct are at first formed by pseudostratified columnar epithelium in which numerous cell divisions are observed, particular in the group of cells situated near the nerve elements of the ganglion spirale. A small Golgi apparatus of elongated shape can be seen in the apical part of the high cells of the epithelium.

The shape of the cross-section of the duct also changes from oval to triangular. These transformations proceed gradually from the basal turn towards the apical turn as the differentiation of the walls of the cochlear duct proceeds: (1) lower wall — tympanic wall with organ of Corti, (2) external wall — *stria vascularis, prominentia spiralis et sulcus spiralis externus,* (3) and the vestibular membrane — Reissner's membrane.

2.1.3. Tympanic wall of cochlear duct. Differentiation of the tympanic wall of the cochlear duct takes place earliest in the area of the basal turn and can be seen in embryos as small as 30 mm. Within the pseudostratified columnar epithelium the cell nuclei are first observed to migrate in the direction of the base of the cells. The hair cells next undergo differentiation, their large round nuclei being visible above the nuclei of the remaining cells of the lower wall (Figs. 12, 15, 16). The cytoplasm of the hair cells stains intensively. These cells have a large oval-shaped Golgi apparatus located above the nucleus (Figs. 18—21).

The space of the future inner tunnel of Corti and Nuel's space begins to form between the pillars. The tunnel of Corti next widens and finally becomes triangular in shape. The formation of this tunnel takes place

along the whole cochlear duct from the basal to the apical turn, the tunnel extending to the epical turn by the time the embryos are 80 mm long. Formation of the supporting cells, pillars, cells of Deiters and cells of Hensen takes place at the same time.

In embryos 55 mm long vacuoles appear in the pseudostratified columnar epithelium, but the cells themselves undergo cytolysis, and *sulcus spiralis internus* is formed. Numerous PAS + grains are observed in the area of the cells subject to cytolysis (Fig. 30). Considerable activity of Ac. Pase can be seen in this place. As a result of cytolysis the pseudostratified columnar epithelium disappears, and the *sulcus spiralis internus* formed is lined with simple columnar epithelium with a flattened Golgi apparatus lying in the apical parts of the cells. These changes take place rapidly and the *sulcus spiralis internus* can already be seen in the area of turn IV in embryos 75 mm in length (Figs. 29, 30, 31, 33, 34).

2.1.4. Tectorial membrane. By the time embryos are 14 mm long a thin tectorial membrane appears in the pericentral part of the tympanic wall on the surface of the pseudostratified columnar epithelium cells. The dimensions of this membrane, which is of gelatinous-fibrous structure and develops from the epithelium cells, gradually increase as differentiation of the cochlear duct proceeds. In embryos 55 mm long, and even more distinctly in embryos 65 mm long, the membrane described above covers the *limbus spiralis*, extends above *sulcus spiralis internus* and above the organ of Corti, with the hair cells of which it remains in close contact (Figs. 31, 33).

2.1.5. *Limbus spiralis*. In embryos 32 mm long a concentration of connective tissue cells forms on the pericentral margin of the cochlear duct, the cells extending concavely into the interior of the wall of this duct. In this way part of the duct epithelium becomes the epithelium covering *limbus spiralis* as it forms. In time the simple columnar cells covering the limbus become closely connected with the stratum and the boundaries of the base of these cells become invisible. *Limbus spiralis* forms two lips — *vestibuli et tympani* (Figs. 12, 15, 21, 31).

2.1.6. Basilar membrane. In embryos 14 mm long, thin reticular fibres appear between the base of pseudostratified columnar epithelium cells of the cochlear duct and the stratum of mesenchymal cells (Fig. 5). As differentiation of the inner ear proceedes these fibres can also be observed within *ligamentum spirale* and *limbus spiralis*. The mesenchymal cells form a stratum lining the basilar membrane from the side of *scala vestibuli*. Blood vessels are located among these cells. Within *zona externa pectinata* of the basilar membrane in embryos 35 mm long two further strata of fibres are faintly marked. Single,

elongated cells with rod-like nuclei can be seen between these two tracts of fibres. In larger embryos the space separating the fibres is more distinct, and the cells located there are slightly larger and more numerous. In embryos 90 mm long the fibres can be seen in *zona pectinata* of the basal membrane in the form of two distinct strata, and in the pericentral part of the basal membrane, in *zona arcuata* — in the form of one stratum (Figs. 5, 23).

2.1.7. External wall. From the time the embryos attain the length of 30 mm an external wall initially formed of pseudostratified columnar epithelium is distinctly visible in the area of turn I. In embryos 35 mm long this epithelium can be seen within the external wall in the form of simple columnar cells, to which connective tissue cells and blood vessels adhere on the exterior. Connective tissue cells and fibres on the exterior of this epithelium form *ligamentum spirale,* assuming a fan-shaped arrangement at the level of *crista membranae basilaris.* Within the epithelium, as in the connective tissue, there are cells with processes, the cytoplasm of which contains grains of brown pigment. The pigment cells are at first visible only outside the ectodermal epithelium. In embryos 55 mm long the following can be distinguished in the external wall: *stria vascularis, prominentia spiralis et sulcus spiralis externus. Stria vascularis* is formed of simple columnar or cuboidal cells, with dark cytoplasm and an invisible boundary in the basal part, between which there are cells with grains of brown pigment. There is one stratum of simple squamous mesothelial cells of connective tissue under the above-mentioned cell layer (Figs. 15, 17, 18, 22, 25—28, 32). Blood vessels occurring at fairly regular intervals adjoin the cells situated near the lumen of the duct. *Prominentia spiralis* is formed of narrow columnar cells, the base of which extends into the interior of *ligamentum spirale.* The cells of *sulcus spiralis externus* lying below communicate with the space of *ligamentum spirale* through a distinct system of canals and concavities.

2.1.8. Vestibular wall. In embryos 32 mm long the epithelium on the site of the forming Reissner's membrane is pseudostratified columnar epithelium. The ectodermal cells of the cochlear duct gradually become flat, and one stratum of mesenchymal cells remains on the side of *scala vestibuli,* while the remainder undergo cytolysis. Thus in embryos 35 mm long we observe Reissner's membrane of two strata of cells. At first the epithelium cells have oval Golgi apparatus, but in larger embryos it becomes distinctly flattened.

Al. Pase, Ac. Pase, S. D. and L. D. are found in cells of Reissner's membrane (Figs. 15, 18, 23, 25, 26, 27, 31).

2.2. First period of development — histochemistry

2.2.1. RNA. During development of the inner ear the cytoplasm of cochlear duct cells and ganglion cells is distinguished by high ribonucleic acid content. During ossification of the cochlear capsule a considerable number of RNA grains are observed in connective tissue cells: pre- and osteoblasts. In embryos 65 mm long Nissl's bodies appear in the cytoplasm of ganglion cells. After the organ of Corti is formed high ribonucleic acid content is visible in the hair cells, *stria vascularis* and *ganglion spirale*.

2.2.2. Glycogen. During formation of the cochlear duct grains of glycogen appear in the apical parts of epithelium cells. In addition diastase-labile grains are present in the mesenchymal cells and cartilage cells. Large amounts of glycogen grains appear transitorily in the cells of *stria vascularis* (Fig. 17) and in the cells of the epithelium of vestibuli of the cochlear duct (tunnel of Corti, cytoplasm of cells within the area of the future *sulcus spiralis internus*). Glycogen grains are not visible in the hair cells and cytoplasm of ganglion cells until the embryos attain the length of 65 mm. The number of glycogen grains in these cells rapidly increases. At the same time quantitative predominance of glycogen is observed in turns III and IV of the cochlea (Figs. 7, 13).

2.2.3. Lipids. In the inner ear fats occur as fats connected with the cytoplasm, cell membranes and myelin sheath of nerve fibres. Neutral fats (glycerides) occur in cytoplasm of cells of Hensen in the form of minute globules, which appear in the area of turn III and IV in embryos 65 mm long (Fig. 36). As the embryos grow the number and size of the globular lipid elements increase in the cells of Hensen. Lipids are transitorily visible on the pericentral side of the organ of Corti in the cytoplasm of cells of pseudostratified columnar epithelium. The myelin sheaths of nerve fibres blacken distinctly with osmic acid (Fig. 36) and stain with scarlet *R*.

2.2.4. Ac. Pase. During differentiation of the cochlear duct the enzyme exhibits considerable activity in the region of the apical parts of the epithelium cells and in the ganglion cells, the maximum activity of the enzyme corresponding topographically to the area of the Golgi apparatus. Increased activity of the enzyme is also observed in the area where *sulcus spiralis internus* and the tunnel of Corti form (Figs. 9, 10).

2.2.5. Al. Pase. During the early period of differentiation of the cochlear duct the activity of the enzyme in the epithelium is slight, but increases as the organ of Corti and *stria vascularis* form. Al. Pase ex-

hibits great activity in the cochlear capsule and the areas of ossification (Figs. 24, 25).

2.2.6. S. D. Succinate dehydrogenase exhibits little activity in the early period of differentiation of the cochlear duct. During the period of greatly advanced differentiation of the morphological structures of the cochlear duct the activity of S.D. increases in the hair cells, the cells of *stria vascularis* and *ganglion spirale* cells.

2.2.7. L. D. Lactic dehydrogenase at first exhibits slight activity in the region of the epithelium of the otic vesicle. The activity of the enzyme markedly increases as differentiation of the cochlear duct

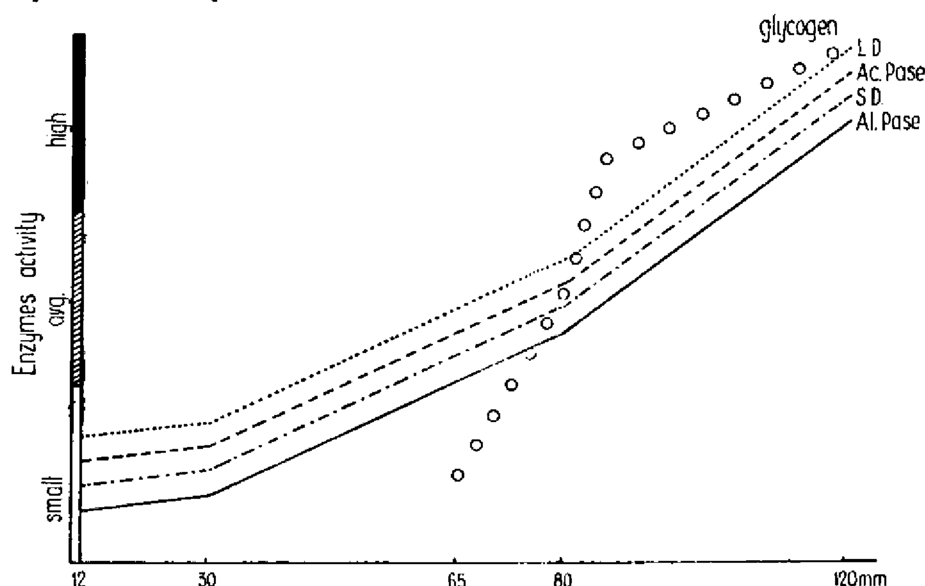

Fig. 49. Activity of enzymes and glycogen contents in hair cells and cells of *stria vascularis* during embryogenesis.

proceeds. The enzyme also occurs in large amounts in the spiral ganglion cells, and far smaller amounts in the mesenchymal cells. In the differentiated cochlear duct considerable activity of L. D. is observed in the hair cells, the cells of *stria vescularis* and the spiral ganglion cells (Figs. 8, 26, 28).

2.3. Second period of development — morphogenesis and histochemistry

The period is characterised in the morphological picture by complete formation of the spaces of *scala vestibuli*, *scala tympani* and complete differentiation of the organ of Corti. The formed tunnel of Corti includes all four turns. This period begins in embryos 80 mm long (Figs. 37, 42, 45, 46, 48).

The activity of enzymes in hair cells, cells of *stria vascularis* and ganglion cells continues to increase (Fig. 41, 43—45, 47, 49). In ganglion cells Nissl's bodies become more numerous (Fig. 38). Glycogen content continues to increase in hair cells and ganglion cells (Figs. 39, 49). The amount of globular lipid elements continues to increase in cells of Hensen (Fig. 40).

The picture described above of the morphological and histochemical differentiation of the inner ear is closely connected with the appearance of the microphonic potential and conditions continued increase in the value of this potential.

V. MICROPHONIC POTENTIAL (*CM*)

In the material elaborated the youngest embryos in which a response was obtained to sounds transmitted by bone conduction were those 80 mm long (Fig. 50). When sounds are transmitted by air conduction,

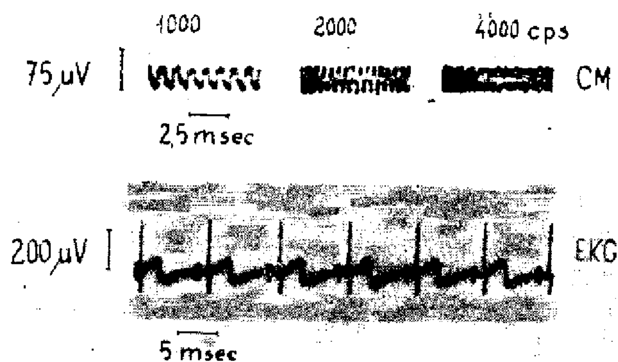

Fig. 50. Cochlear microphonic potential at tones of 1000, 2000, 4000 c.p.s. and 75 db in a guinea pig foetus 80 mm long (upper tracing). *EKG* in foetus (lower tracing).

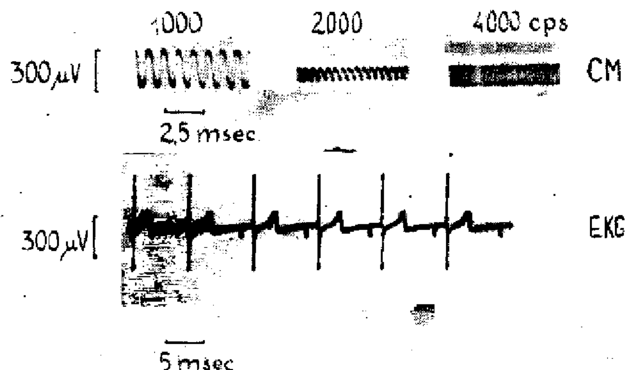

Fig. 51. Cochlear microphonic potential at tones of 1000, 2000, 4000 c.p.s. and 75 db in the guinea pig foetus 110 mm long (upper tracing). *EKG* in foetus (lower tracing).

after the method given by Brohm (1962; 1963), no electric response was obtained from the embryos examined to tones of 1000, 2000 and 4000 c.p.s. at 75—80 db.

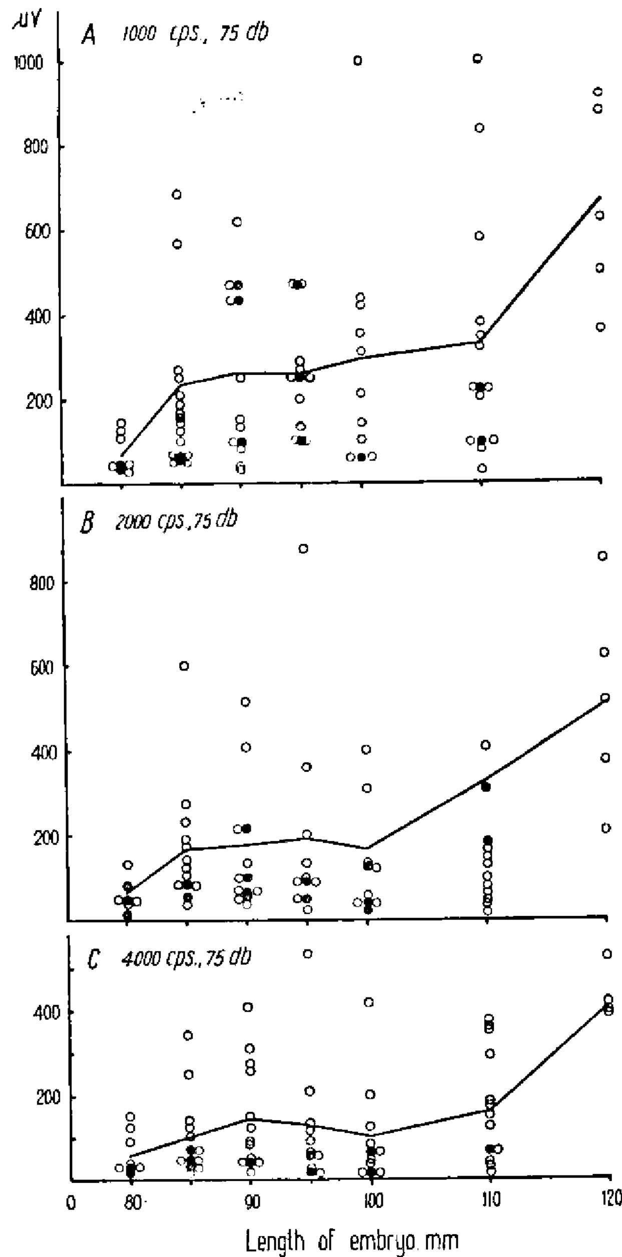

Fig. 52. Values of CM depending on the length of foetuses. A — 1000 c.p.s. 75 db, B — 2000 c.p.s. 75 db, C — 4000 c.p.s. 75 db.
Open circles — one individual, hatched circles — two individuals, points — three individuals.

As the embryos grow the *CM* value increases (Table 1, Figs. 51, 52). This increase is particularly distinct with the use of the 1000 c.p.s. tone; when 2000 c.p.s. and 4000 c.p.s. are used the *CM* values in some groups of embryos undergo transitory reduction. When 1000 c.p.s. tone is used the maximum *CM* values are obtained in the majority of embryos; it is only in the group of embryos 80 mm long that the maximum *CM* values are not distinctly characteristic of the 1000 c.p.s.

Table 1.
The values of *CM* in guinea pigs foetus in μV.
Observed ranges, averages and standard errors are given.

Foetus length in mm.	Number of experiments	1000 c.p.s.	2000 c.p.s.	4000 c.p.s.
80	9	33 — 140 70 ± 13.8	21 — 130 58.1 ± 10.5	9—140 56.1 ± 14.6
85	14	75 — 650 221.7 ± 43.5	39 — 600 157.2 ± 37.0	12 — 330 93.3 ± 23.0
90	13	35 — 600 248 ± 51.4	42 — 500 180.5 ± 41.5	12 — 390 139.3 ± 33.2
95	11	97.5—450 243 ± 34.1	30 — 850 181.7 ± 68.5	18 — 510 118 ± 40.1
100	11	50 — 1000 277 ± 76.0	18 — 400 159 ± 42.1	9 — 400 93.3 ± 32.4
110	14	24 — 1000 307.3 ± 72.9	15 — 390 156.2 ± 25.8	9 — 360 153.3 ± 34.0
120	5	350 — 900 636 ± 98.6	200 — 820 496 ± 94.2	250 — 500 384 ± 34.0

tone (Fig. 53). In the groups of oldest embryos (110—120 mm long) *CM* values are high — they attain several hundred μV and are remarkably close to the values recorded for adult animals.

VI. GENERAL DISCUSSION

The formation of the auditory part of the inner ear (cochlea) may be regarded as a process about which we possess a good deal of information owing to the histological methods now in use. These methods, however, do not provide a unequivocal explanation of the complicated processes taking place in the cells and tissues during the formation of the inner ear, and on this account histochemical methods and the electrophysiological method are used in addition to histological methods.

The results obtained will be compared with the observations of other authors in studies on the embryogenesis of the organ of hearing in different animals, in the following order: (1) morphogenesis of the inner ear in the guinea pig, (2) histochemistry of the development of the inner ear in the guinea pig, (3) electrophysiology.

1. Morphogenesis

During the formation of the cochlea in the guinea pig the cochlear duct increases in length and at the same time assumes a spiral arrangement. This increase is accompanied by differentiation of the duct,

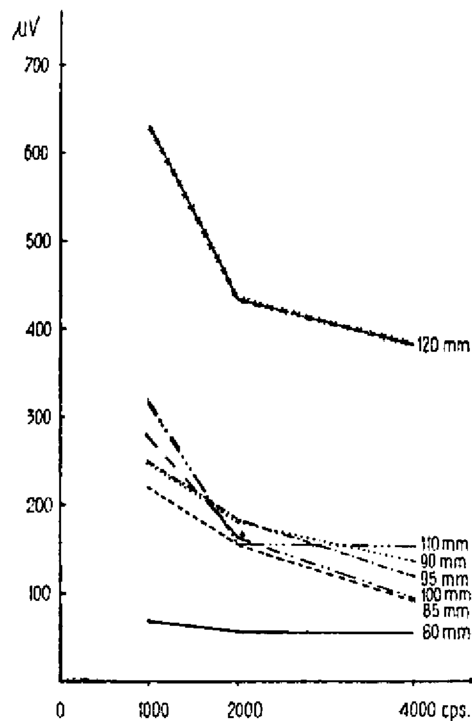

Fig. 53. Curves showing the mean values of *CM* depending on frequency of tones (1000, 2000, 4000 c.p.s.) in foetuses of different lengths.

proceeding from the basal turn upwards. Development of this kind was defined by K o l m e r (1927) as particularly characteristic of the development of the cochlear duct in general, and by W e i b e l (1957) in relation to development of the cochlear duct in white mice.

B é l a n g e r (1956), however, during his studies on the development of the inner ear in mice, observed earlier differentiation of the apical turn in comparison with the lower-lying turns of the cochlea. Similarly

Ruben (1967), on the basis of the time of occurrence of mitoses in cells of the developing inner ear in the mouse, describes the earlier differentiation of the organ of Corti in the apical turn, and the later differentiation in the basal turn.

Formation of connective tissue structures: the spiral ligament, basilar membrane and *limbus spiralis* takes place at the same time as the differentiation of the duct. These structures, according to the studies made by Mangabeira-Albernaz (1961), play an active part in the biochemical transformations of the inner ear and undergo change as the result of the action of intensive sounds. The osseous labyrinth forms on the outside of the membranous labyrinth (Bast, 1944; Cabrini, 1961; Costa & Covell, 1967). Of the walls of the cochlear duct morphological differentiation is visible earliest in the lower wall. In guinea pig embryos as small as 32 mm long two ridges can be discerned in this wall (Figs. 12, 15): in the lateral ridge (the smaller) the hair cells form later on, while the cells of *sulcus spiralis internus* are located in the larger (pericentral). A thin membrane appears on the surface of the cells of the two ridges, which later becomes the tectorial membrane (Figs. 15, 17, 33). Hardesty (1915) considers that the tectorial membrane of pig embryos is formed only on the surface of the cells of the large pericentral ridge. Similarly Weibel (1957) considers that the fibres of the tectorial membrane in mice are formed chiefly by cells of the internal spiral sulcus. Naftalin *et al.* (1964) demonstrated by means of chemical methods the similarity of the proteins from which the fibres of the tectorial membrane in cats are built to the epithelial proteins. Policard & Baud (1958), like Iurato (1960; 1962), consider the fibres of the tectorial membrane as keratinomyosynofibrins. Borghesan (1959), who examined the tectorial membrane in guinea pigs, rabbits and human embryos, emphasises the close morphological connection between this membrane and the organ of Corti, which was also observed in preparations of guinea pig embryos (Fig. 33). De Vries (1949) also refers to the connection between the tectorial membrane and the hair cells.

The differentiation of the cells of the organ of Corti has formed the subject of many studies. As has been shown, the differentiation process in the lower wall in the guinea pig begins with the separation of the hair cells, which differ from the other cells of the cochlear duct as to shape, capacity for staining and location. In Vinnikov's opinion (1959) the receptor cells of the inner ear in vertebrates appear during phylogenetic and ontogenetic development when anaerobic glycolysis and oxygen respiration are sufficiently developed. Weibel (1957), on the other hand, considers that the supporting system is formed from

the beginning together with differentiation of the hair cells. In Titova's opinion (1965) there are two stages in the development of the organ of Corti: (a) morphological and biochemical differentiation of the neurons of the spiral ganglion and receptor cells (b) differentiation of the supporting cells.

In the guinea pig, as in many other animals, formation of the supporting cells takes place below the hair cells (Figs. 12, 15, 31). The following are formed: tunnel of Corti and space of Nuel. The question of the formation of these tunnels remains open to discussion. Van der Stricht (1920) connected the formation of these spaces with the process of cytolysis in the supporting cells. Weibel (1957) considers that these spaces are formed as the result of secretion of vacuoles by the cells. These spaces next widen as the result of the separation of the lower parts of the supporting cells together with increase in the dimensions of the basilar membrane.

The internal spiral sulcus forms on the pericentral side of the organ of Corti. The cells of the pseudostratified columnar epithelium lose contact with the tectorial membrane, undergo cytolysis and only the cuboidal cells remain (Fig. 29). There are grains of glycogen in the cells of this epithelium (Fig. 30). Weibel (1957) and Titova (1965) both refer to the vacuolisation of this epithelium, Weibel (1957) finding granulation in the cells — which might also have been grains of glycogen. On the strength of histological investigations and measurement of *CM* it may be assumed that formation of the internal spiral sulcus on all the turns would appear to be an essential condition for the bioelectric functioning of the organ of Corti.

The spiral prominence forming the external wall of the duct and *stria vascularis* are, in the opinion of Vosteen (1960) and Chou (1961) the site of active biochemical metabolism connected with processes of oxygenisation and secretion. The cells of *stria vascularis* are also, in the opinion of Davis (1957; 1962) and Tasaki (1959) the source of the endocochlear potential. The ectoderm epithelium and the mesenchymal cells participate in the formation of the external wall (Kolmer, 1927; Weibel, 1957; Grisanti, 1957; Kikuchi & Hilding, 1965). In guinea pig embryos 35 mm long cells with processes appear, the cytoplasm of which contains grains of brown pigment. At first these cells are visible outside the layer of ectoderm epithelium, but as the embryos grow the grains of pigment are also encountered in the cells within *stria vascularis* (Figs. 15, 32). According to the studies made by Engström *et al.* (1955) two types of cells can be distinguished in *stria vascularis* of the guinea pig: chromophobe

and chromophil, the chromophobe cells being considered to be of mesodermal origin and contain grains of pigment. Beck (1961) hold that the pigment grains take part in metabolism of the cochlear duct. Kikuchi & Hilding (1966) consider that the structure of the cells of *stria vascularis* in the mouse during development of the ear points to the capacity of these cells to secrete fluid. In his studies of the development of the ear in the guinea pig the author observed great enzyme activity in the cells of stria and the presence of PAS + grains, which points to the activity of this structure in the process of secretion as early as the period of embryonic life, since secretion in the cells of *stria vascularis* is connected with intensive metabolism (Vosteen, 1956). Grisanti (1957) on the other hand, who examined rabbit embryos, denies this possibility, since he found the blood vessels in *stria vascularis* to appear fairly late and an absence of close contact between these vessels and the cells. Borghesan (1965) describes the system of canals situated below *prominentia spiralis*, which system, in the author's opinion, is intended to exercise a secreting function before the final formation of *stria vascularis*. These spaces are also active in adult animals (Vosteen, 1957; Hilding, 1965; Chodynicki, 1965).

The vestibular wall — Reissner's membrane develops from the cells of the roof of the cochlear duct and from the mesenchymal cells situated on the side of *scala vestibuli*. According to the description given by Kolmer (1927) the bistrate epithelium of the pericentral side becomes first of all double-rowed, then single-rowed and finally-flat. Reissner's membrane is finally formed of two layers of flat cells in close contact, but which can be distinguished under a microscope. In preparations of guinea pig embryos, according to this author both the layers have Golgi apparatus, and ash pictures contain numerous minute mineral particles in Reissner's membrane (Figs. 15. 18, 25—27). Lactic dehydrogenase (L.D.) and alkaline phosphatase (Al. Pase) are also present in the cells of Reissner's membrane, which in Rauch's opinion (1966) points to the activity of the membrane while maintaining balance in the composition of fluids present between the space of *scala vestibularis* and the space of the cochlear duct.

2. Histochemistry

In the material elaborated the histochemical picture of the inner ear underwent change during the course of its development. During the initial period of development it is connected with the process of formation and differentiation of the structures of the inner ear, and

the intensive histochemical transformations in turn precede the bioelectric function of the organ of Corti.

According to Brachet (1964) RNA plays an important part in the differentiation process, it participates in protein synthesis and also induces the development of morphological structures. Great increase in RNA content is observed primarily in the areas of intensive production of proteins, and thus in the embryonic tissues (Rodkiewicz *et al.* 1956). As differentiation of the organs proceeds, however, RNA content tends to decrease (Oshiro & Perlman, 1965). The role of DNA and RNA in the differentiation process is also emphasised by Titova (1965) in her histochemical studies on the development of the organ of Corti. During his investigations of the development of the inner ear in the guinea pig during the first period of differentiation the author observed high RNA content in the cytoplasm of the cochlear duct cells; during the period immediately preceding function of the organ of Corti the number of RNA grains in the cytoplasm of ganglion cells increases. The marked basophil character of the ganglion cells is also emphasised by Bélanger (1956). Nucleic acid content in the hair and ganglion cells alters under the influence of sounds (Vinnikov & Titova, 1961; 1963; Chodynicki, 1962).

Glycogen occurs in the cochlear duct cells of the guinea pig as early as the initial period of differentiation, and later can be seen in the cells of *stria vascularis* and the supporting cells (Figs. 7, 13, 17). During formation of the tunnel of Corti and spaces of Nuel in guinea pigs numerous glycogen grains can be seen, similar to the grains encountered during formation of *sulcus spiralis internus* (Fig. 30). According to Falben-Hansen (1967) glycogen in the developing inner ear is synthetised from proteins, its content in the ear structures being subject to change. In the hair and ganglion cells glycogen appears during the period preceding function of the organ of Corti and the glycogen content in these elements rapidly increases (Fig. 39). According to Vinnikov (1957) glycogen in hair cells plays an important part in anaerobic metabolism. Titova (1965) observed coincidence between the appearance of glycogen in hair cells and the development of dark, rod-like mitochondria connected with anaerobic metabolism. Vosteen (1964) describes the close connection of glycogen grains with the membranes of mitochondria in cells of *stria vascularis* and nerve endings. The cells of the labyrinth in lower vertebrates contain a large amount of glycogen (Vinnikov, 1959), but PAS + grains are not encountered in the external hair cells of bats (Plotz & Perlman, 1965). Glycogen content in hair cells decreases under the influence of sounds (Zorzoli

& Boriani, 1958; Vinnikov & Titova, 1961; Chodynicki, 1962; 1965). The author considers that the distribution of glycogen in the cells of the inner ear during embryogenesis would seem to point to its trophic significance during differentiation of the cochlear duct, and that it next participates in the energy metabolisms when function of the organ of Corti begins.

During embryogenesis of the inner ear in the guinea pig (Chodynicki, 1966), in addition to lipids connected with cytoplasms, cell membranes and the myelin sheath of fibres, neutral fats appear in Hensen's cells in the area of turns III and IV (Fig. 36). These fats in Hensen's cells at first accumulate in the form of small globular elements increasing in volume as the embryos grow. Lipids are transitorily observed on the inner side of the organ of Corti; they may constitute trophic material for the organ of Corti (Fig. 40). This view coincides with Borghesan's assumption (1965) that the organ of Corti is nourished by the cells of the large ridge during its development. Rauch (1964) attributes the role of store substance to lipids. The globular lipid elements, in addition to the role of store substances, may also play the part of shock absorbers on account of both their localisation and properties (Chodynicki, 1966).

In addition to nucleic acids, enzyme processes would appear to play an important part in differentiation of the inner ear. Among the enzymes examined it is acid phosphatase (which as is known, participates in the processes of lysis and pinocytosis and occurs in lysosomes), which exhibits great activity during the first period of embryogenesis.

The participation of acid phosphatase in cytolytic processes can be clearly seen, particularly during formation of *sulcus spiralis internus*.

Ishii & Balogh (1966) defined the activity of Ac. Pase in the ear of guinea pig embryos and in an adult guinea pig, but did not observe any great differences in the behaviour of the activity of this enzyme. In the study made by these authors Ac. Pase occurred in the cells of *stria vascularis*, in the cells of *sulcus spiralis*, Deiters' cells and in the supranuclear space of internal hair cells and the subcuticular space of external hair cells. The authors emphasise a certain relation between the activity of Ac. Pase and accumulation of indissoluble products of cell metabolism. The enzyme activity in the hair cells is held to decrease under the influence of sounds (Vinnikov & Titova, 1963). The author's own observations show that the localisation of Ac. Pase in the cells of the inner ear corresponds to the area of the Golgi apparatus (Figs. 9, 10, 45). During development of the inner ear this apparatus is large and characteristic of the various cells. Vorbrodt (1967) considers that the large Golgi apparatus encountered

in embryonic cells may participate in the formation of lysosomes. Towards the end of embryonic life the Golgi apparatus is slightly smaller (with the exception of the ganglion cells) and difficult to discover by cytological methods. Iurato (1962) recorded the presence in cells of the inner ear in young rats of numerous mitochondria, ribosomes and a well-developed Golgi apparatus. He connects these particularly well developed cytoplasm structures with protein synthesis. Similarly Kikuchi & Hilding (1965) observed in cells of the inner ear in newborn white mice numerical predominance of mitochondria, the Golgi apparatus, ribosomes and endoplasmic reticulum in comparison with adult animals (Spoendlin, 1957; 1959; Chevance & Maduro, 1961).

As shown by the material elaborated, the activity of Al. Pase is at first connected with the cochlear capsule — the enzyme occurs in the perichondrium, and later in the centres of ossification (Figs. 24, 25, 41). The activity of the enzyme next increases in the cytoplasm of the hair cells, ganglion cells and cells of *stria vascularis*, which is connected with active cell transport. Dorfman & Epsztejn (1950) observed increased activity of Al. Pase during the process of differentiation of the neural tube, and connect this increase with protein synthesis. Vinnikov (1958) who examined the enzyme in the inner ear of adult animals, assumes that Al. Pase participates in synthesis and decomposition of glycogen.

In hair cells and cells of *stria vascularis* during embryogenesis the author found constant increase in the activity of succinate dehydrogenase and lactic dehydrogenase connected with mitochrondrial fraction of cells (Figs. 26, 28, 43, 44). According to Miętkiewski & Łukaszyk (1967) activity of dehydrogenase increases as differentiation of the nephrons of chicken kidneys proceeds. This phenomenon is accompanied by the formation of new mitochondria. L. D. also occurs in the nerve fibres in the vicinity of the hair cells (Figs. 43, 44). Titova (1965) connects the appearance of oxydising enzymes and mitochondria in the nerve endings with the start of functioning of the hair cells. Increased activity of L. D. and S. D. is particularly distinct during the period preceding bioelectric functioning of the organ of Corti connected with transformation of acoustic energy into nerve impulses. According to Vosteen (1956) the source of the energy for these processes is oxygen metabolism, in which cytochromeoxidase and succinodehydrogenase participate. The activity of the enzyme processes decreases after the longer action of sounds (Vosteen, 1960, 1961; Vinnikov & Titova, 1961, 1963; Chodynicki, 1962). When the energy sources of oxygen metabolism decrease this deficit may be supplemented by

anaerobic metabolism with the participation of L. D. (Rauch, 1964). Increased enzyme activity (succinate dehydrogenase and cytochromeoxidase) occuring at the moment when the cells assume the characters of adult cells, was observed in the cerebral cortex of pigs by L. and J. Flexner (1946). Similar observations in relation to different enzymes were made by Wawrzyniak (1966) in his studies on the development of the mesencephalon in guinea pigs.

On the strength of the author's own investigations and the observations of other authors it may be assumed that the activity and localisation of enzyme processes are characteristic in different periods of embryogenesis. During the first period they are connected with differentiation of the organ of hearing. The activity of enzyme and energy processes within the newly-formed structures gradually increases with the growth of the embryos and precedes the receptor function of the inner ear. The second period — the period of function of the organ of Corti — is characterised by a histochemical picture similar to the picture of the inner ear in adult animals. These facts make better understanding of the significance of the development of the inner ear possible.

3. Electrophysiology

An interesting problem is that of the capacity of the organ of hearing to receive sounds during the period of embryonic life (Dębowski, 1950; Carmichael, 1957). The morphological and physiological studies made so far are concerned with the development of the organ of hearing in animals during the first days *post partum*.

For instance McCrady, Wever & Bray (1940) and Larsell, McCrady & Larsell (1944) on the basis of a number of studies made on the opossum (*Didelphis virginiana*) which lives in the mother's pouch after birth, did not find reaction of the organ of hearing to sounds until the 48th day after birth. At first the inner ear of opossum reacts only to a certain range of frequency, which widens as the animal matures. The excitability threshold, which during the first few days after birth is high and is not lowered until later, is also subject to change. Similar observations were made on opossums by Schmidt & Fernandez (1963) in defining the endocochlear potential: this potential appeared in animals on the 25th—30th days after birth, and values characteristic of adult animals were attained on the 78th day. In white mice and rats, however, the potential appeared shortly before birth and attained maturity on the 14th day. Alford & Ruben (1963) found that in white mice the cochlear microphonic potential appeared earliest 11.6 days after birth, and the potential of auditory

nerve VIII (12.5 days) and Preyer's reflex (most often on the 12th day) slightly later. The organ of Corti in histological preparations of the animals examined was completely formed. Mikaelian & Ruben (1965) also found Preyer's reflex to depend on the functional maturity of nerve VIII and that there is an interdependence between anatomical development of the organ of hearing and the physical reaction and behaviour of the animals. The authors in addition express the view that histological methods are insufficient to explain the causes of the absence of physiological reactions in a certain number of the animals.

Kljavina & Maruseva (1963) recorded nerve potentials in cats at the age of 2—30 days. They registered distinct response in animals on the 9th day after birth and that only when considerable intensivity of the excitations was applied. Mazo (1955) defined the electrical response of the organ of hearing in bird nestlings and newborn rabbits to tones of different frequency. This author considers that function of the organ of hearing develops parallel to development of the whole organism, but at a different time in different species of animals.

Änggärd (1965) in his studies on the development of cochlear function in the rabbit also found increase in *CM* value with age and widening of the range of frequencies, both in the direction of low and high tones, received by the maturing organ of hearing. An increase in the value of the endocochlear and summating potential was also observed. The above studies refer to animals outside the mother's organism, but we know less about the function of the organ of hearing during its embryonic development.

According to Dębowski (1950) all parts of the guinea pig embryo function as from the very early stages of development. The animal possesses full capacity for exercising its organs, and early function of parts of the embryo is an important factor in development. A different opinion in relation to the organ of hearing in man was presented in Godlewski's Embryology (1948): neither the embryo nor the newborn child are considered to be able to receive sound impressions. In guinea pig embryos 80 mm long, in which the organ of Corti is completely formed, the author obtained a response to sound in the form of microphonic potential (Fig. 50). Bornschein & Krejci (1953) consider this potential as the most objective criterion of function of the inner ear in animals. According to Davis (1954; 1957; 1959; 1961; 1962) the microphonic potential is supposed to form in the external hair cells and in addition to the summating potential serves as a mediator between mechanical bending of the hairs and initiation of the nerve impulse on the second pole of the hair cells. This is closely

connected with nervation of the hair cells (Engström, 1958; Wersäll, Hilding & Lundquist, 1961). In the opinion of several authors: Spoendlin (1957; 1959), Rydberg (1960), Iurato (1961), Engström *et al.* (1962), Flock *et al.* (1962) the process of formation of electrical impulses is closely connected with the function of organellae and cell metabolism. The value of the microphonic function in the guinea pig embryos examined increases almost evenly with the age of the embryo and before birth attains several hundred μV (Table 1, Figs. 51—53). These results agree with the observations of other authors who examined animals during the first few days after birth (Mazo, 1955; Schmidt & Fernandez, 1963; Änggärd, 1965; Kljavina & Maruseva, 1965). There is thus a certain analogy in the development of the organ of hearing in the guinea pig during embryonic life in comparison with the development of this organ in other animals during the first days *post partum* (opossum, mouse, rabbit).

In the guinea pig embryos examined the highest microphonic response was mainly obtained with a tone of 1000 c.p.s. (Fig. 53), with the exception of embryos 80 mm long, in which the predominance of the values noted at 1000 c.p.s. is less marked. To a certain extent the differences in microphonic response may be connected with the case of change in the shape of the skull and shift of the vibration system within the skull of the embryos, and may also be due to the immaturity of the organ of hearing (Table 1). Results of measurement of microphonic potentials in embryos also depend to a great degree on the state of placental circulation: we observed this relation in our previous investigations made on guinea pig embryos (Chodynicki & Matwijewicz, 1966, 1967, 1968). Bone conduction is also connected with the physical properties of the vibrating systems, frequency of vibrations and place on which the strength acts (Fournier, 1953; Ranke, 1953; Békésy, 1963; Bystrzanowska, 1963).

To recapitulate it may be assumed that the factor integrally connected with formation of the organ of hearing and forming the basis of the receptor function of this organ consists of histochemical processes, increase in activity of biochemical processes corresponding to increase in the value of microphonic potential. The *CM* measurements made point to the possibility of the organ of hearing in guinea pig embryos receiving sounds. These studies agree with the clinical observations on the reaction of human embryos to sounds, expressed by acceleration of heart function or encephalographic recording (Brown, 1964; Dwornicka, 1963; Elliot, 1964; Johansson, 1964).

Acknowledgments: The author has pleasure in expressing his gratitude to Dr. H. Lewińska, Head of the Department of Histology and Embryology of the Białystok Medical School, for the assistance and valuable advice unfailingly given during the preparation of this publication. It is also his pleasant duty to thank Professor W. Hassmann, for his valued help and Professor Z. Kanigowski, for the facilities provided for making measurements in the Electrophysiological Laboratory of the Neurological Clinic. Thanks are also due to technical assistans: Miss. M. Stachowska and Mrs. B. Dolistowska.

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Received, February 17, 1968.

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EXPLANATION OF PLATES

PLATE IV.

Fig. 1. Axial section of embryo 12 mm long, showing the otic vesicle and endolymphatic duct. HE. Magn. 7.5×.

Fig. 2. Cross-section of otic vesicle and endolymphatic duct. Length of embryo 12 mm. HE. Magn. 53×.

Fig. 3. Golgi apparatus in cells of otic vesicle and endolymphatic duct. Length of embryo 12 mm. Aoyama's method. Magn. 53×.

Fig. 4. Axial section through inner ear of embryo. Reading from right the following can be seen: the space of the forming *bulla tympanica*, then section of cochlear duct, part of the saccule and utricle, endolymphatic duct and the vesicle of the semicircular canal lying above it. Length of embryo 14 mm. HE. Magn. 23×.

Fig. 5. Cross-section of cochlear duct. Reticular fibres can be seen in the vicinity of the duct. Length of embryo 14 mm. Laguess's method. Magn. 90×.

Fig. 6. Cochlea in axial section. Length of embryo 17 mm. HE. Magn. 53×.

Fig. 7. PAS + granules in cells of cochlear duct. Turn II. Length of embryo 17 mm. PAS. Magn. 184×.

Fig. 8. Lactic dehydrogenase in cells of cochlear duct and ganglion cells. Length of embryo 20 mm. Hess's method. Magn. 64×.

PLATE V.

Fig. 9. Acid phosphatase in cells of cochlear duct and ganglion cells. Length of embryo 20 mm. Gomori's method. Magn. 113×.

Fig. 10. Acid phosphatase in cochlear ganglion cells. Length of embryo 20 mm. Gomori's method. Magn. 113×.

Fig. 11. Ash picture. Cochlear duct at level of turn II. Length of embryo 20 mm. Magn. 64×.

Fig. 12. Hair cells, can be seen in central part of lower wall of cochlear duct, turn I. Length of embryo 32 mm. Gallocyanin. Magn. 225×.

Fig. 13. Axial section of cochlea. PAS+ granules in cells of cartilage, connective tissue and cochlear duct in area of turn IV. Length of embryo 32 mm. Magn. 22×.

Fig. 14. Golgi apparatus in nerve cells of ganglion spirale. Length of embryo 32 mm. Aoyama's method. Magn. 825×.

Fig. 15. Cross-section of cochlear duct with distinct differentiation of the various walls. Hair cells, can be seen in central part of lower wall, turn II. Length of embryo 35 mm. HE. Magn. 94×.

Fig. 16. External hair cells, external tunnel, Hensen's cells, turn II. Length of embryo 35 mm. Azan. Magn. 1800×.

PLATE VI.

Fig. 17. PAS+ granules in cells of external wall of cochlear duct, turn I. Length of embryo 35 mm. PAS. Magn. 94×.

Fig. 18. Golgi apparatus in cells of cochlear duct, turn II. Length of embryo 35 mm. Aoyama's method. Magn. 94×.

Fig. 19. The same preparation. Golgi apparatus in hair cells. Magn. 1801×.

Fig. 20. The same preparation. Golgi apparatus in cells of pseudostratified columnar epithelium of lower wall of cochlear duct. Magn. 1800×.

Fig. 21. The same preparation. Golgi apparatus in cells of *limbus spiralis* Magn. 1800×.

Fig. 22. Axial section of cochlea. Length of embryo 38 mm. Azan. Magn. 22×.

Fig. 23. Reticular fibres in basilar membrane, turn II. Length of embryo 38 mm. Gomori's method. Magn. 225×.

Fig. 24. Alkaline phosphatase in ganglion cells. Length of embryo 40 mm. Comori's method. Magn. 450×.

PLATE VII.

Fig. 25. Alkaline phosphatase within turn II of cochlea. Length of embryo 50 mm. Gomori's method. Magn. 64×.

Fig. 26. Lactic dehydrogenase in cells of cochlear duct, turn II. Length of embryo 50 mm. Hess's method. Magn. 64×.

Fig. 27. Ash picture of cochlear duct, turn II. Length of embryo 50 mm. Magn. 113×.

Fig. 28. Lactic dehydrogenase in cells of *stria vascularis* and hair cells, turn II. Length of embryo 55 mm. Nachlas's method. Magn. 150×.

Fig. 29. Lower wall of cochlear duct. Cytolysis is visible in cells of pseudostratified columnar epthelium, turn III. Length of embryo 55 mm. HE. Magn. 315×.

Fig. 30. PAS+ granules in cells undergoing cytolysis, turn II. Length of embryo 55 mm. PAS. Magn. 225×.

Fig. 31. Lower wall of cochlear duct with organ of Corti differentiating: hair cells and pillars can be seen: turn I. Length of embryo 65 mm. Azan. Magn. 188×.

Fig. 32. External wall of cochlear duct. *Stria vascularis, prominentia spirais*, with *ligamentum spirale* and the cochlear capsule with areas of ossification below, can be seen. Length of embryo 65 mm. Azan. Magn. 263×.

PLATE VIII.

Fig. 33. *Membrana tectoria*. Hairs can be seen on the surface of the hair cells, turn II. Length of embryo 65 mm. Azan. Magn. 450×.

Fig. 34. Golgi apparatus in cells of *sulcus spiralis internus*, turn III. Length of embryo 65 mm. Aoyama's method. Magn. 450×.

Fig. 35. Golgi apparatus in cells of ganglion spirale. Length of embryo 65 mm. Aoyama's method. Magn. 825×.

Fig. 36. Lipids in Hensen's cells and in sheaths of fibres of nerve VIII. Isolated turn III. Length of embryo 65 mm. Osmic acid. Magn. 100×.

Fig. 37. Cross-section through cochlea. Length of embryo 85 mm. HE. Magn. 12×.

Fig. 38. Cells of ganglion spirale at level of turn IV. Length of embryo 85 mm. Brachet's method. Magn. 825×.

Fig. 39. PAS+ substance in external and internal hair cells. Isolated turn IV. Length of embryo 85 mm. PAS. Magn. 218×.

Fig. 40. Lipids in Hensen's cells and *sulcus spiralis internus*. Isolated turn IV. Lenght of embryo 85 mm. Oil red O. Magn. 94×.

PLATE IX.

Fig. 41. Alkaline phospatase in external hair cells. Isolated turn II. Length of embryo 85 mm. Gomori's method. Magn. 225×.

Fig. 42. Two strata of fibres within external part of basilar membrane. Length of embryo 90 mm. Gomori's method. Magn. 450×.

Fig. 43. Lactic dehydrogenase in hair cells. Isolated turn IV. Length of embryo 100 mm. Hess's method. Magn. 24×.

Fig. 44. The same preparation. Enzyme can be seen in internal hair cells and nerve endings. Magn. 218×.

Fig. 45. Acid phosphatase in hair cells, cells of Claudius, cells of *stria vascularis;* turn III. Length of embryo 100 mm. Burstone's method. Magn. 90×.

Fig. 46. Cross-section of organ of Corti, turn III. Length of embryo 120 mm. Osmic acid. Magn. 188×.

Fig. 47. Succinate dehydrogenase in hair cells. Isolated turn IV. Length of embryo 120 mm. Nachlas's method. Magn. 24×.

Fig. 48. Golgi apparatus in ganglion cells. Length of embryo 120 mm. Aoyama's method. Magn. 825×.

Stanisław CHODYNICKI

EMBRIOGENEZA CZĘŚCI SŁUCHOWEJ UCHA WEWNĘTRZNEGO ŚWINKI MORSKIEJ

Streszczenie

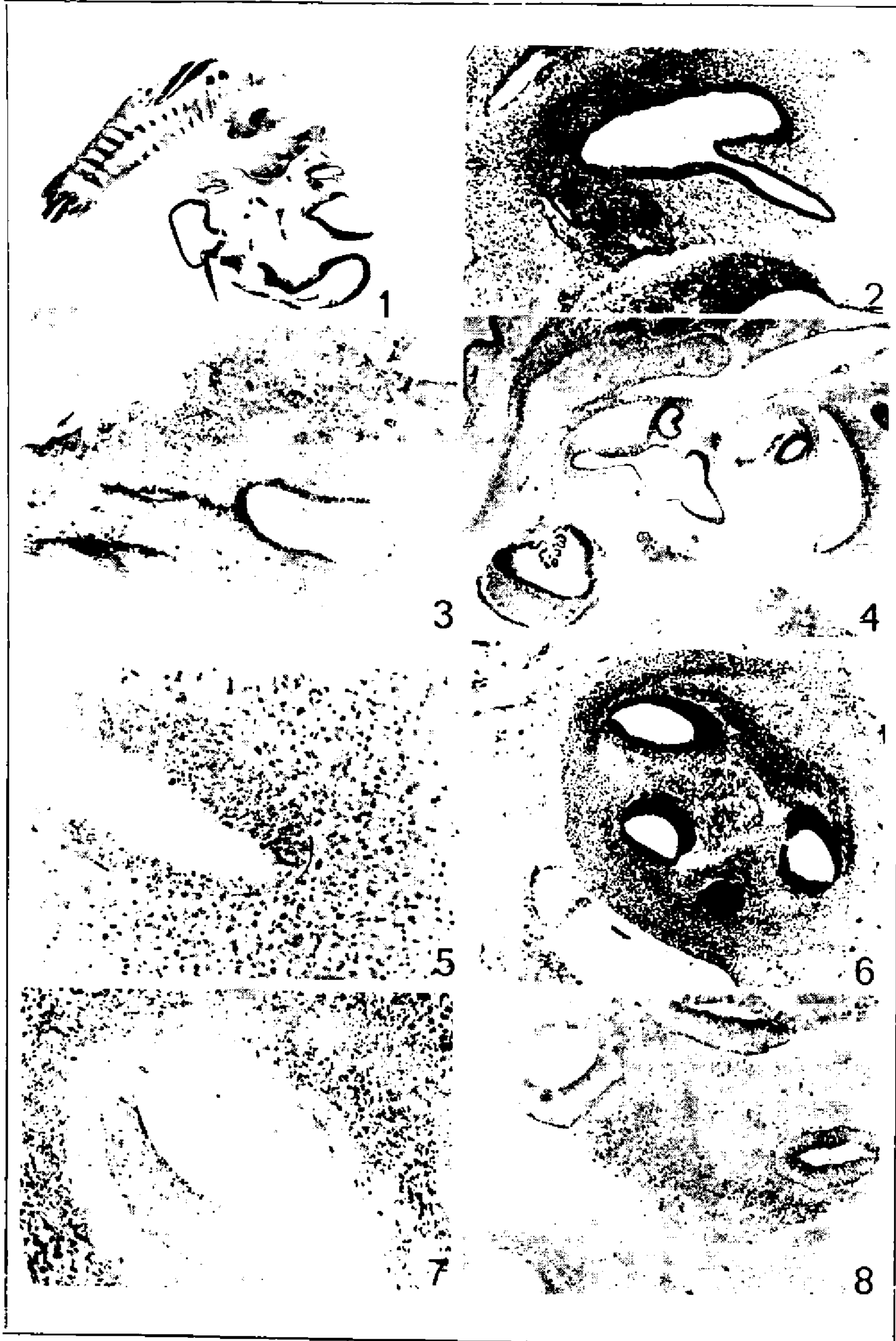
Praca obejmuje badania histologiczne, histochemiczne oraz pomiary potencjału mikrofonicznego wykonane w czasie rozwoju ucha wewnętrznego u płodów świnki morskiej. Badano płody w różnym wieku, a ich długość określano w wymiarze ciemieniowo-ogonowym. Preparaty ucha wewnętrznego sporządzano techniką mroże-

niową, parafinową oraz metodą preparowania powierzchniowego. Materiał barwiono HE, met. Azan, wykrywano aparat Golgiego, kwasy nukleinowe, polisacharydy, lipidy oraz enzymy: dehydrogenazę kwasu bursztynowego i kwasu mlekowego, fosfatazy: kwaśną i zasadową. Począwszy od płodów długości 80 mm dokonywano pomiaru potencjału mikrofonicznego z zastosowaniem przewodnictwa kostnego przy tonach 1000, 2000, 4000 Hz i 75 db. Rejestrowano również czynność serca. Pomiary te wykonywano w warunkach zachowanego krążenia łożyskowego.

Stwierdzono, że podczas morfologicznego kształtowania ucha wewnętrznego w komórkach i tkankach zachodzą charakterystyczne przemiany histochemiczne. Tak np. największą zawartość kwasów rybonukleinowych w komórkach przewodu ślimaka obserwowano w pierwszym okresie embriogenezy. W dalszym rozwoju cytoplazma komórek była znacznie uboższa w RNA. W miarę różnicowania się przewodu ślimaka i zwoju spiralnego zwiększa się aktywność enzymów w komórkach włoskowych, komórkach prążka naczyniowego i komórkach zwojowych oraz pojawiają się w tych komórkach polisacharydy.

Zauważono, że w różnych okresach embriogenezy poszczególne komórki przewodu ślimaka posiadają charakterystyczny aparat Golgiego. Np. w komórkach zwojowych aparat Golgiego położony jest początkowo biegunowo, a począwszy od płodów długości 65 mm przyjmuje położenie apolarne.

Już u płodów 80 mm długości, u których narząd Cortiego jest już wykształcony na przestrzeni całego przewodu ślimaka, stwierdzono odpowiedź ucha wewnętrznego na dźwięki w postaci potencjału mikrofonicznego. Wartość potencjału mikrofonicznego, określana w μV, wzrasta w miarę wzrostu płodów i też przed urodzeniem osiąga wartość kilkuset μV. Czynność bioelektryczna narządu Cortiego jest poprzedzona wzrostem intensywności przemian biochemicznych w komórkach zmysłowych ucha wewnętrznego.



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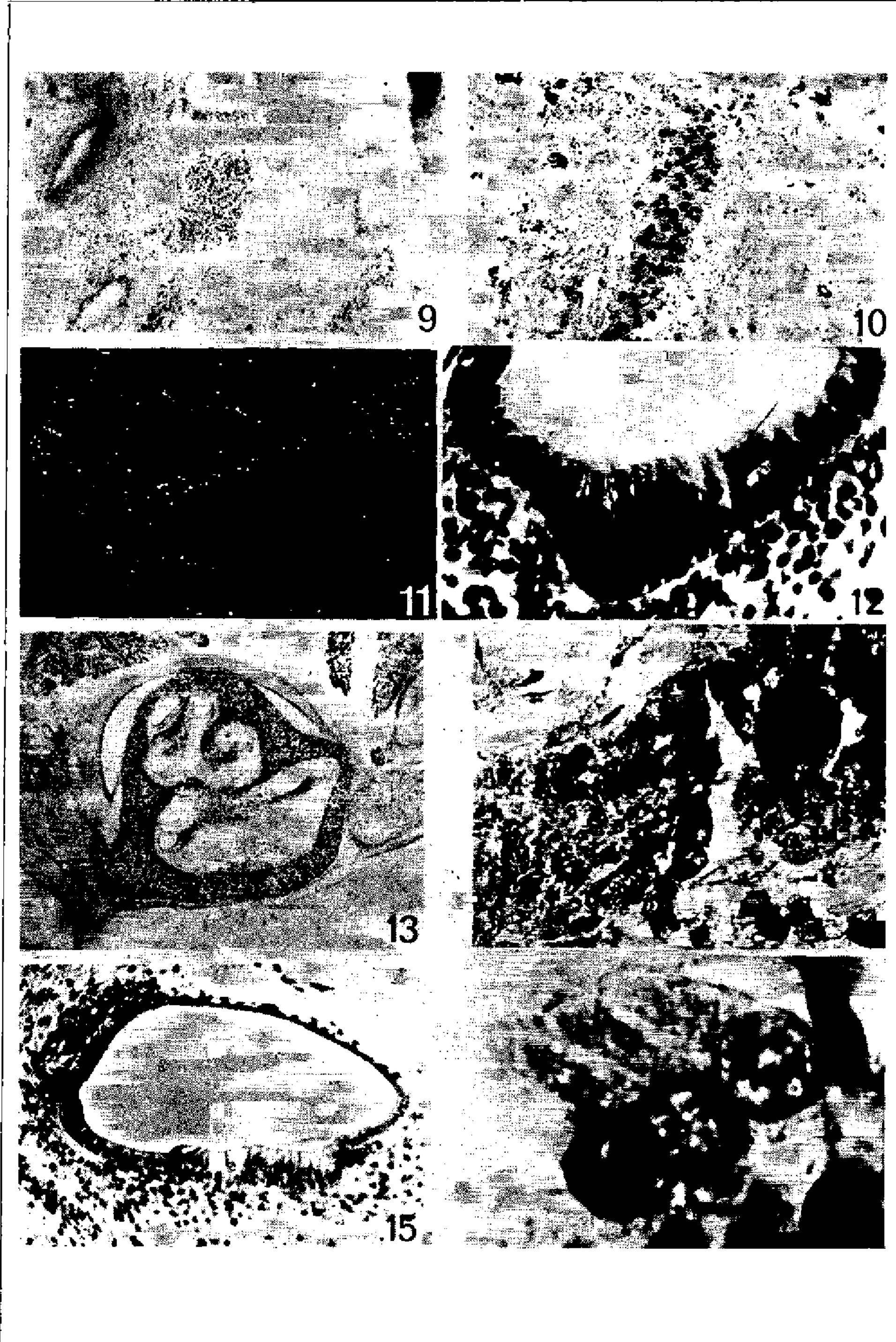

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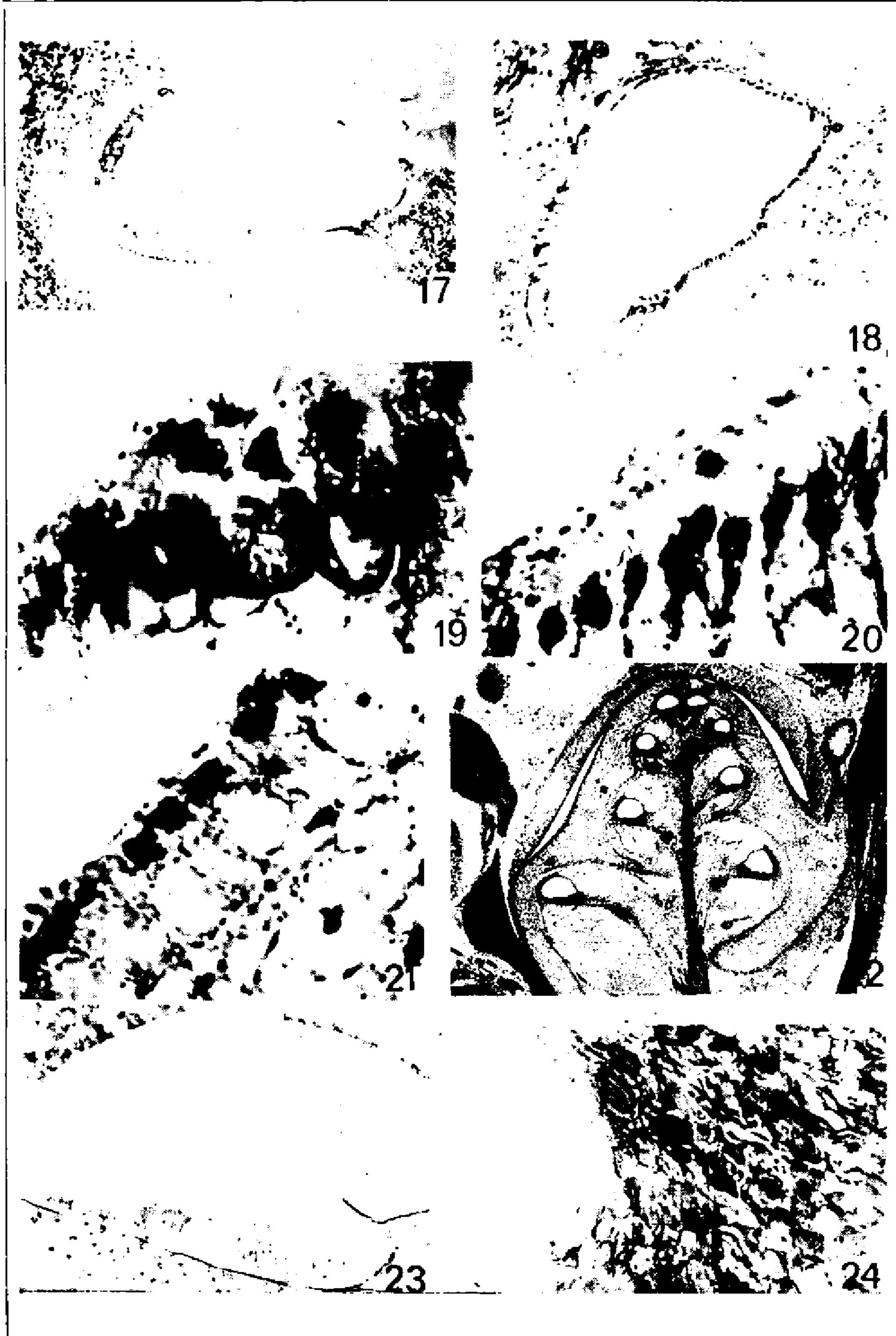

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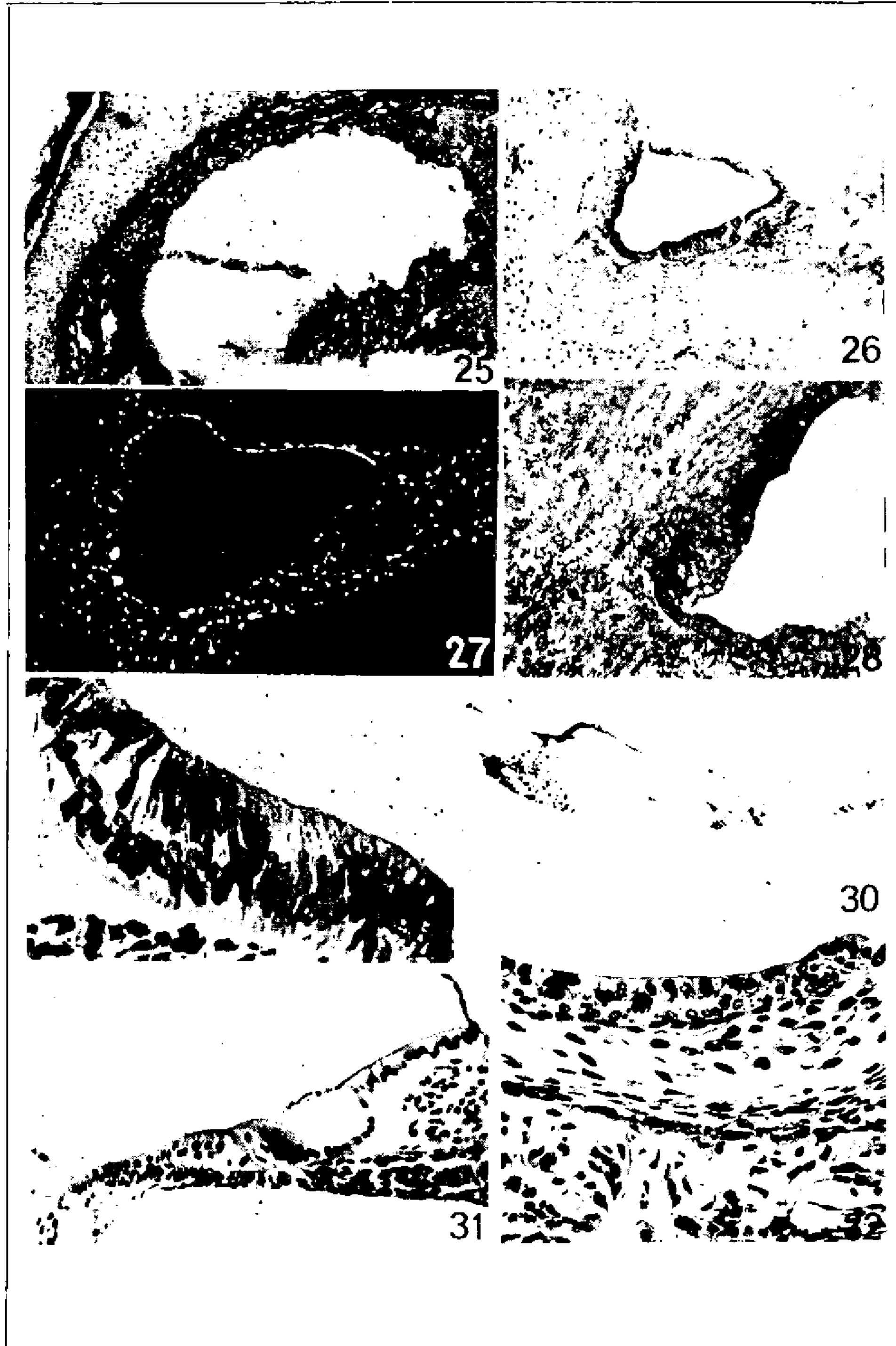

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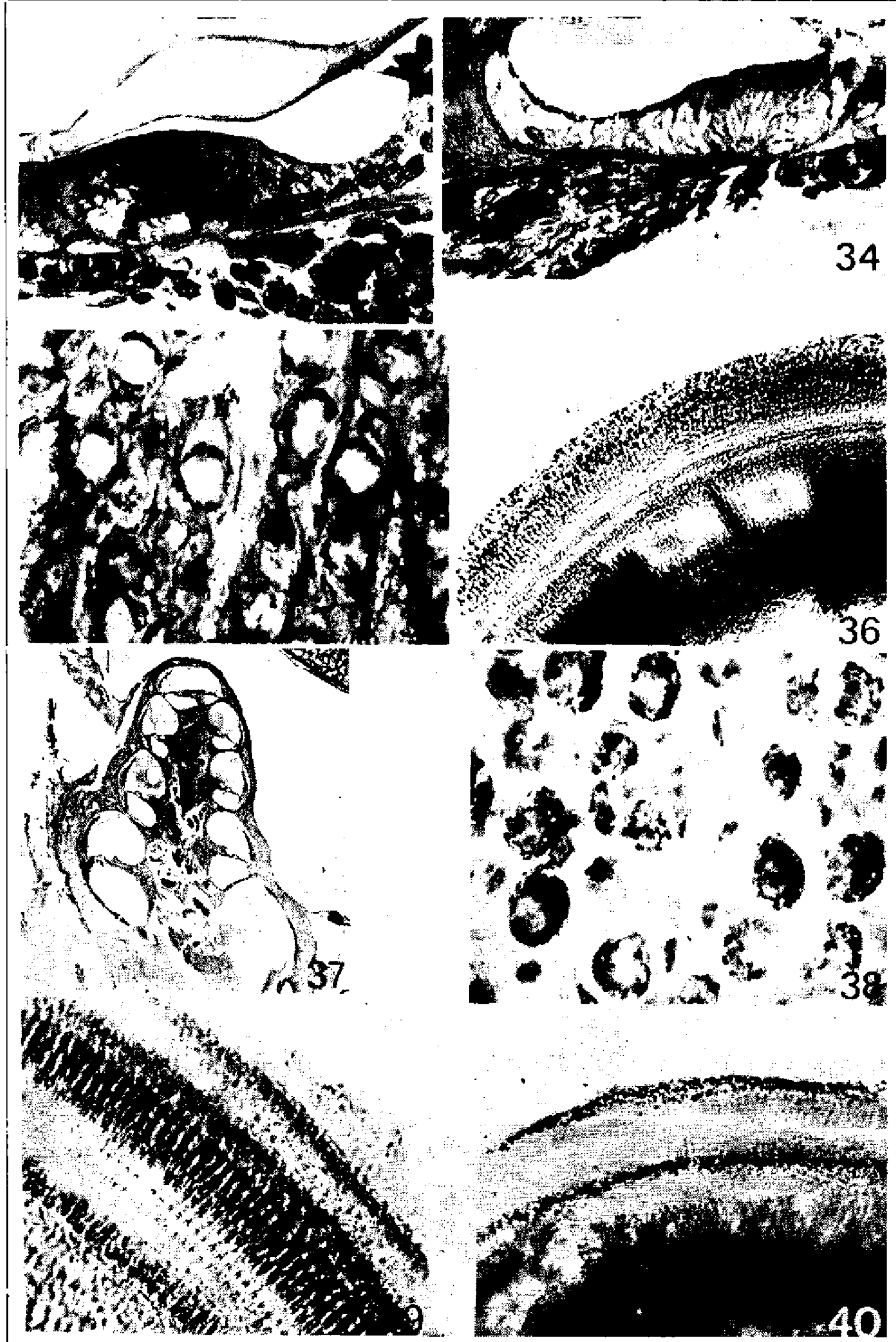

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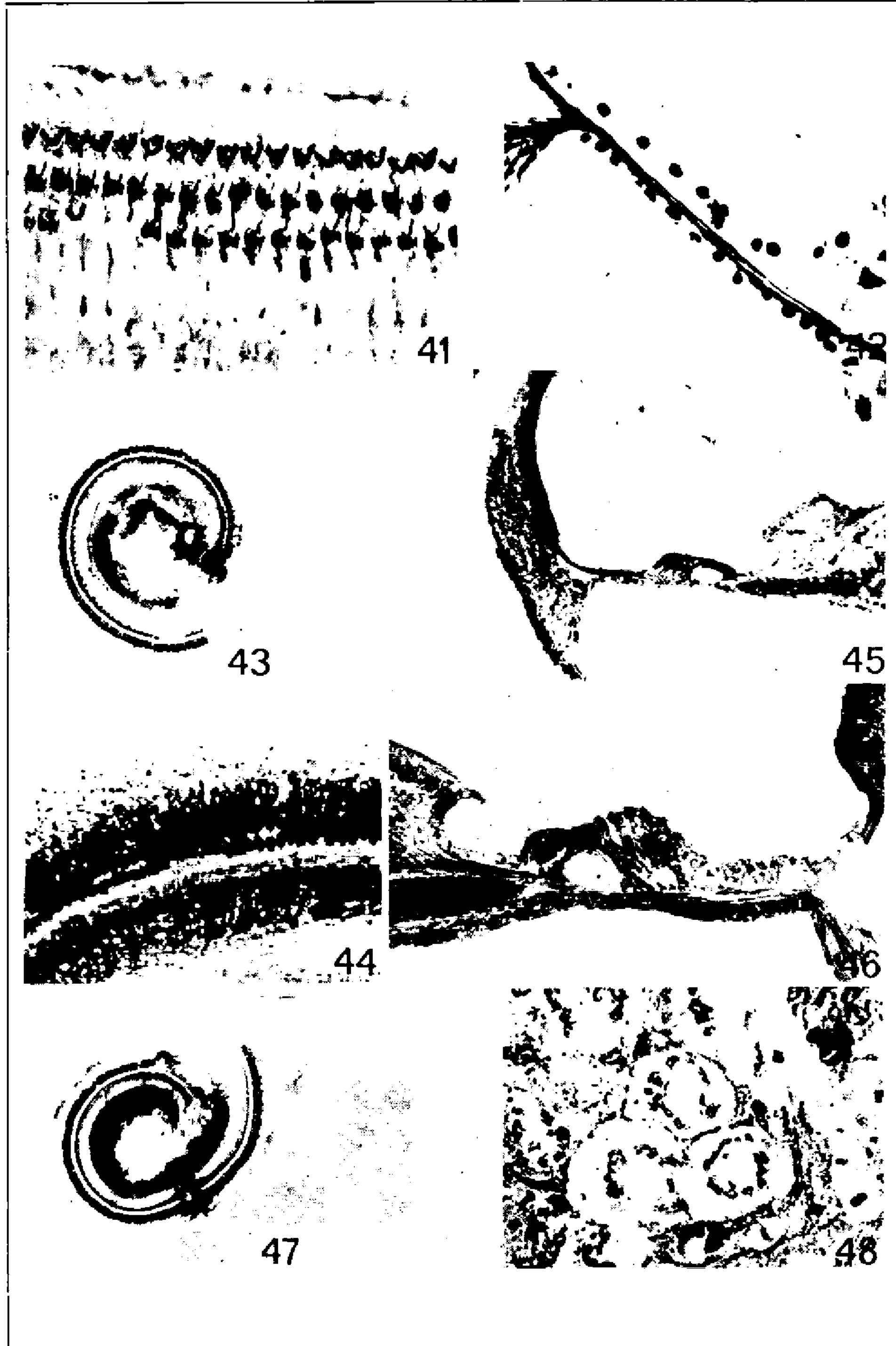

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