The Larval Ear of the Frog and its Transformation During Metamorphosis*

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The evolution of the higher vertebrates. In branchiates like Proteus, Necturus etc., the change from fish to tetrapod is faithfully repeated, generation after generation. An aquatic larval period precedes the adult terrestrial period. The change from one to the other is more extensive and complete in some orders and families than in others. Perennibranchiates like Necturus and Proteus, retain throughout their life, a prevalingly piscine organization. Various larval characters persist also in the common newts and salamanders, so that their metamorphosis really is incomplete. Frogs and toads go through the most extensive changes and after metamorphosis, approach the amniote type. Their locomotor and respiratory organs become entirely remodeled. After metamorphosis they develop new nutritional habits, and accordingly also the digestive system suffers radical changes. In fact, almost the entire body becomes rebuilt, with the exception of the sex glands, which are little affected and attain maturity often only after metamorphosis.

In view of the general, physiologic and evolutionary importance of metamorphosis in the frog, it is surprising that it has never been investigated and described in a systematic and monographic way. One of the many organs that have not received due attention is the ear. Yet the functional change from underwater to aerial hearing is accompanied by replacement of the larval accessory apparatus with the entirely new tympanic organ of the adult.

In the present paper the description of the metamorphosis of the ear is preceded by a short outline of the developmental history of some of the other structures which enter in a relationship with the auditory organ. The following numbering system will be used to characterize the successive larval and metamorphic stages:

St. 21 Hatching larvae, external gills.
St. 22 — St. 24 Non-feeding larvae, gill sac formation.
St. 25 Larvae with closed gill sacs, feeding begins, round limb buds.
St. 26 — St. 29 Larval stages with progressively differentiating hind legs.
St. 30 — St. 33 Stages of metamorphosis.
St. 34 Young Frogs.

Outline drawings of several of these stages are given at the upper right hand corners of the figures 1—6.

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The pulmonary system which is to supplement and eventually replace the branchial respiratory organ begins to develop very early. In larvae ready to hatch (stage 21) the floor of the pharynx forms a narrow depression which is the laryngotracheal rudiment. From its distal part arise two lateral diverticula, a seventh pair of pharyngeal pouches. They are the primordia of the paired bronchi and lungs.

During the larval period, the lungs serve as hydrostatic as well as respiratory organs. Like the swim bladders of fishes, they tend to adjust the specific gravity of the entire body to that of the water and are used to shift the gravitational center. The respiratory surface becomes increased in the distal parts by the formation of internal septa, Thus form the alveolar lung sacs (fig. 3).

The developmental relationship between branchial and pulmonary organs expresses itself also in the detail of their innervation and vascularization. Like the last three branchial arches, the lungs are supplied by the vagus nerve. Pulmonary arteries and pulmonary veins are the afferent and efferent vessels of a seventh aortic arch (figs. 1, 3) and the pulmonary capillary plexus takes the place of a branchial network. The blood flows dorsoventrally, that is, opposite to the direction of that taken in the four branchial arches. This is a consequence of the fusion of the ventral seventh arch with the atrium of the heart, where the blood pressure is lower than in the arterial duct. In the perennibranchiate Necturus, a sixth aortic arch does not develop; nevertheless the pulmonary vessels form as in other amphibians, proving the independence of the seventh from the sixth arch. The origin of the pulmonary circuit near the dorsal aorta and the dorsoventral direction of its blood flow, resemble the conditions in fishes, where the swimbladders are supplied by vessels from the dorsal aorta.

The fifth to the seventh of the aortic arches make changing connections among themselves. When the sixth arch appears (frog embryos of about 7 mm), it branches off from the fifth and again returns to it. Later its efferent vessel makes a connection with the last appearing, seventh or pulmonary arch. The common short vessel through which the sixth and seventh communicate with the dorsal aorta is the arterial duct (figs. 1, 2). During the late larval period, the dorsal root of the fifth arch shifts caudally and eventually also connects with the arterial duct (fig. 4). The cutaneous artery originates from the dorsal segment of the sixth arch.

At metamorphosis the involution of the gills begins shortly before the arms break through the gill sacs. It keeps about the same pace as the resorption of the tail. Together with the gills, disappear also the marginal gill muscles (which accompany the afferent branch of the aortic arches in the larva), and large parts of the branchial cartilages (compare figs. 1 and 6). When the resorption of the tail is complete the gills, gill slits, and gill sacs have also vanished, except for fibrous remnants and pigment masses.

During early metamorphosis (stages 30—31) the larval organization of the vascular system is still essentially intact (cf. figs. 1—3). Rapid changes begin concurrent with the regression of the gills and the tail (stages 32—33; fig. 4). The blood of the ventral aortae is suddenly forced into the efferent limbs of the aortic arches by way of pre-existing pores in the interaortic septa (see below). Subsequently the afferent limbs shrink and disappear (fig. 4).

Special conditions develop at the root of the third arch. At the time of the development of the larval branchial plexus, the direct communication between aortic trunk and ventral carotid was throttled through the development of the thick interaortic septum of the third arch. From then on the ventral carotid was supplied from the gills by way of the efferent branch of the aortic arch (fig. 1). However the septum always retained some potential anastomoses. They are forced open at metamorphosis, when the gill capillaries close and regress. Thus the ventral carotid becomes again a direct branch of the aortic trunk. At the same time a prominent swelling develops in the root of the carotid arch (dorsal carotid); this is the carotid body (figs. 5, 6). Sections show that it consists of a porous diagonal membrane, and a sponge-like body in the median wall of the vessel. The main current of blood flows through the pores, while a smaller amount seeps through the dense pad. The interaortic septa of the fourth and fifth arches disappear without leaving remnant struc-
tures (fig. 5). The literature on this subject was reviewed in the article by Pischinger 1.

Up to stage 31, the efferent limbs of all arches open into an uninterrupted dorsal aorta, where free passage exists in both the rostral and the caudal directions. At the climax of metamorphosis (fig. 4, stage 32) the fifth arch undergoes a rapid reduction, starting with the constriction of its ventral root and occlusion of its dorsal segment. A similar reduction of the dorsal part of the sixth arch brings about the transformation of the arterial duct into an arterial ligament. The proximal part of the sixth arch persists and serves as the common root of the cutaneous and the pulmonary arteries.

The adult condition becomes fully established during the late stages of metamorphosis (stages 33, 34; figs. 5, 6). The fifth arch and the arterial ligament disappear completely. The dorsal aorta between the third and the fourth arches becomes reduced to a fibrous, persisting aortico-carotid ligament. This completes the development of separate carotid (3) and systemic (4) arches.

**Ossification**

Bone formation is not entirely a characteristic of metamorphosis. Ganoids and teleosts have bones structurally similar to those of terrestrial salamanders and frogs, which proves that in certain groups the evolution of ossification preceded the advent of the tetrapods. As a rule urodeles which after metamorphosis become terrestrial, have more heavily ossified skeletons than their perennibranchiate relatives. While in salamanders bone formation begins at hatching and slowly progresses throughout the larval period, in frogs it occurs mainly during metamorphosis 2. In recent amphibians the cartilaginous skeleton becomes only partly replaced by bones. In frogs the main structure of the chondrocranium persists; its cartilage merely becomes fortified, and locally replaced, through perichondral bone formation (figs. 4—6).

**Visceral Apparatus**

The visceral apparatus becomes radically reconstructed. The larval arrangement of cartilages (figs. 1, 2) and muscles, besides serving branchial respiration, also satisfies the particular requirements of detritus eaters. But the metamorphosed frog subsists entirely on live prey. Accordingly, the very small mouth of the tadpole changes into the extremely large one of the frog.

In the mandibular arch region, transformation starts with the shedding of the horny teeth (stage 31, fig. 2). Then the quadrate cartilage with the articular process rotates ventrally around the point of its attachment at the ear capsule. At stage 33 (fig. 4) it projects at right angles from the body of the skull and later it slants decidedly in the caudal direction (fig. 5). In the meanwhile, the attachment to the orbitonasal lamina is preserved; this involves a considerable stretching of the connecting pterygoid process of the quadrate (figs. 4, 5). The lower jaw grows in length at the same rate as its joint with the quadrate moves caudally. At the end of metamorphosis, it is about six times as long as it was at the start.

These changes are accompanied by progressive ossification (cf. figs. 4—6). In the upper jaw, the place of the rostral cartilages is taken by the premaxillary bones. On the quadrate three separate ossifications appear: maxilla, pterygoid, and squamosal. The latter is T-shaped and the frontal arm of its cross bar, the zygomatic process, develops on the cartilage which in the larva (fig. 2) was the muscle process of the quadrate. A clasp develops laterally around the masticating muscles, through the outgrowth of a caudal process from the maxilla, and through the rostral extension of the quadrato-maxilla, a bone that appears on the lateral surface of the articular process of the quadrate. The quadratomaxilla finally forms a major part of the upper jaw. The pterygoid, ossification of the pterygoid process of the quadrate, curves medially around the masticating muscles, and becomes included in the roof of the oral cavity. In the lower jaw, the rostral cartilages fuse with the mandibles (pars mentalis) and both parts become covered by two bones dentary and angular. An extensive study of the transformation of the mandibular arch was published by Pusey 3.


The cartilages of the second to the sixth visceral arches fuse into one broad plate which is embedded in the floor of the pharyngeal cavity and in the root of the tongue. Current designations like hyoid or hypobranchial cartilage for this structure are misleading, the former already being in use for one part of the composite structure, the latter wrongly implying that it includes only branchial cartilages. Instead the author proposes to rename it as the pharyngeal cartilage. Its paired lateral processes are derived from the second to the sixth visceral arches (figs. 5, 6). The hyoid process grows to a considerable length and takes the shape of a slender rod, which curves caudally around the tympanic cavity and attaches itself to the otic capsule near the oval foramen. On the contrary, the distal parts of the branchial cartilages melt away until only short processes and ridges remain. They still serve for the attachment of muscles which formerly controlled the movements of the branchial apparatus and now assume analogous functions in the buccopharyngeal respiratory system. The last process poses to rename it as the pharyngeal cartilage. Its paired lateral processes are derived from the second to the sixth visceral arches (figs. 5, 6).

The muscles which attach themselves to the pharyngeal cartilage are either visceral or somitic in origin. Of the former, the four petrophyaryngeal (petrohyoid) muscles are derived directly from the levators of the branchial arches. Dorsally, they start from the parotic crest of the ear capsule and their contractions lift the floor of the pharyngeal (oral) cavity. The first one is supplied by the glossopharyngeal nerve; the other three receive branches of the vagoles. Below the last of the branchial levator muscles and belonging to the same series, follows the laryngeal levator, a muscle of the seventh visceral arch (figs. 5, 6). The muscles which attach themselves to the pharyngeal cartilage are either visceral or somitic in origin. Of the former, the four petrophyaryngeal (petrohyoid) muscles are derived directly from the levators of the branchial arches. Dorsally, they start from the parotic crest of the ear capsule and their contractions lift the floor of the pharyngeal (oral) cavity. The first one is supplied by the glossopharyngeal nerve; the other three receive branches of the vagoles. Below the last of the branchial levator muscles and belonging to the same series, follows the laryngeal levator, a muscle of the seventh visceral arch. At metamorphosis the center of this muscle becomes attached to the lowest process (VI) of the pharyngeal cartilage; the dorsolateral part disappears, and the ventromedial portion transforms into the laryngeal musculature of the adult frog. The laryngeal nerves are branches of the vagus nerve. The strong levator of the hyoid arch, the orbitohyoid, is a purely larval muscle and disappears during metamorphosis.

Most important of the somitic muscles, are the sternohyoid and the geniohyoid, which may be considered as terminal segments of the abdominal rectus muscle. Contractions of the former lower the floor of the oral cavity. Both are supplied by the second spinal nerve (which also contains the remnants of the first and some fibers from the third). Like the branchial levators, these longitudinal muscles are little changed in the metamorphic process.

**Ear**

A new investigation of the development of the membranous labyrinth of *Rana temporaria* and of *Triton alpestris* was published by B i r k m a n n. The anatomic studies of E c k e on the ear of the toad *Bufo vulgaris* are also of interest.

The otic vesicle or otocyst forms in late neurula and early tailbud embryos (stage 16—17) by invagination of the otic placode. At stage 18 it is a hollow sphere with two distinct prominences; one is a remnant of the attachment of the placode to the neural layer of the skin; the other, at the top of the vesicle, is a new formation, the rudiment of the endolymphatic diverticulum. Neuroblasts separating from the medial wall of the vesicle aggregate into a clubshaped mass, which becomes the acoustic ganglion.

In larvae with external gills (stages 21—24) the endolymphatic diverticulum consists of a terminal swelling and a narrow duct which opens into the otic vesicle through a pore in the dorsomedial wall. Nerve fibers grow from the acoustic ganglion into the rhombencephalon, forming the roots of the eighth nerve. About the time of closure of the gill sacs (stage 24—25) the saccule appears as a small lobe at the ventral pole of the otocyst. The much larger remainder is the utricle. It is of irregular shape, with depressions and septa that indicate the beginning of the formation of the semicircular canals. The endolymphatic duct opens into the saccule at the base of its connection with the utricle (fig. 3).

In larvae of stage 26 (about twice the length of 25, buds of hind legs about four times as long as wide), the semicircular canals begin to rise
Figure Explanations

Small scale outline drawings characterizing the developmental stage are placed at the upper right hand corner of the figures. Arrows point to the location of the quadrato-mandibular joint.

Blue: cartilage (all figures).
Green: endolympathic system (fig.3), partly also visible through the cartilaginous otic capsule (figs. 2, 4, 5).
Yellow: endodermal structures (figs. 1–6).
Yellow: perilymphatic system (fig. 3).
Yellow: ossifications (figs. 4–6).

Fig. 1. Frog larva (*Rana clamitans*), stage 26; visceral skeleton, heart, and vascular system of the pharyngeal region; ventral aspect. The atrium (dark) mostly hidden by the ventricle of the heart. The aortic trunk branches into four pairs of aortic arches (3–6, afferent and efferent vessels). Interaortic septa near the ventral roots of the third, the fourth, and the fifth arches; afferent and efferent limbs of the sixth arch communicate through small anastomoses. Paired thyroid glands, on the basal plates of the branchial cartilages. Two pairs of parathyroids, at the ventral roots of the fourth and fifth aortic arches. Paired ultimo-branchial bodies (close to the heart) remnants of the sixth pharyngeal pouch. The endodermal lining of the oral cavity and the first pharyngeal pouch with tympanic rudiment are only shown on the left side (right in figure). The lung sacs are not inflated. 12/1.

Figs. 2, 3. *Rana clamitans* in early metamorphosis (stage 31); horny teeth of lower jaws completely lost, those of the upper jaw partly. Lateral view (fig. 2) and rear view (fig. 3) of chondrocranium, ear with bronchial and tympanic accessory organs, and parts of circulatory system. Fig. 2 includes only the proximal part of the right lung, in fig. 3 both lung sacs are displaced ventrally, exposing their dorsal aspects. The right cutaneous arteries are cut off near the root of the pulmonary artery (on the 6th aortic arch). 10/1.

In fig. 2, a part of the wall of the right dorsal aorta is cut away to show the bronchial columella and its attachment to the round window. The tympanic columella ends in a mesenchymatic cord that follows the course of the facial nerve.
In fig. 3, one sees the right endolymphatic (green) and perilymphatic (yellow) systems exposed through removal of a part of the chondrocranium of this side. In reality, the perilymphatic duct is wider than here indicated. Note its contacts with the basilar and the amphibian papillae of the sacculus (in the drawing below endolymphatic duct and below ampulla of posterior semicircular canal resp.). The bronchial columella and the perilymphatic duct are broadly attached to the opposite surfaces of the round window. Endolymphatic and perilymphatic sacs penetrate into the cranial cavity. Through the foramen magnum, the cut end of the medulla is visible. The pharynx is cut off at the level of the glottis. The right bronchial membrane is removed, through the hole appears the ventral wall of the bronchus.

Fig. 4. Rana clamitans during metamorphosis (stage 32); chondrocranium and early ossifications; articular process of the quadrate and tympanic cavity and the ring cartilage are differentiating and have attained a considerable size. The plectrum, distal segment of the columella, lies in a notch of the cartilage and projects toward a fold in the wall of the tympanic cavity; aortic arches in full transformation from larval to adult condition. The bronchial columella has disappeared. 10/1.

Fig. 5. Frog (Rana clamitans) at the end of metamorphosis (early stage 34); lateral view of cranium and visceral skeleton (I—VI, cf. fig. 6) with ossifications, heart, and aortic system. The soft parts of the tympanic organ have been removed, but one can easily visualize the endoderm lined tympanic cavity; it is of conical shape with the round base located in the tympanic ring and the apex passing through the space (+) between otic capsule, squamosal and hyoid process (II) into the oral cavity; 3, 4, 6 persisting aortic arches (cf. fig. 6). 10/1.

Fig. 6. Frog (Rana clamitans) at the end of metamorphosis (early stage 34); ventral view of visceral skeleton, heart, and roots of the major arterial vessels and endocrine glands. I mandibular arch, II hyoid process of pharyngeal cartilage, III—VI branchial processes of pharyngeal cartilage, VII laryngotracheal cartilage; 3 dorsal carotid, 4 systemic arch, 6 common root of pulmonary and cutaneous arteries. Endocrine organs of left side: thyrroid, two parathyroids, ultimo-branchial body. 10/1.
above the utricle. On the saccule a further diverticle, the lagena becomes noticeable. The entire organ has now reached a stage which corresponds to the terminal condition in fishes. The endolymphatic sac is considerably enlarged and lies within the cranial cavity, closely applied to the rhombencephalon. The otic vesicle is enclosed in a capsule which becomes partly cartilaginous while the larva grows from stage 25 to stage 26. In the medial wall of this otic capsule remain several openings, among them the endolymphatic foramen through which the endolymphatic duct passes into the cranial cavity.

In the further development, the otic vesicle becomes the membranous labyrinth (fig. 3). An excessive enlargement of the endolymphatic sacs is peculiar to the amphibians. In salamanders they are confined to the extradural space of the cranial cavity, but in the frogs they extend also into the vertebral canal*. Left and right sacs fuse extensively. Nothing definite is known about the functional significance of the sacs which remain in permanent communication with the labyrinths. Calcareous deposits are most notable in the chalci sacs around the spinal ganglia and, as otoliths, in various parts of the auditory vesicles.

Although in some fishes the perception of mechanical vibrations is refined to such a degree that physiologically one may speak of a sense of hearing, the evolution of the cochlea, the acoustic organ of the mammals, starts only in the amphibian class. A series of new parts appear during the larval and metamorphic stages:

a) Two new diverticles of the saccule, the amphibian and the basilar papillae (fig. 3). The first one is found in all amphibians but not in the amniotes. The second, still lacking in some primitive perennibranchiates, is always present in salamanders and frogs. It is this latter which in reptiles and birds develops into a prominent appendage, and becomes the cochlea in the mammals. The two papillae are supplied by separate branches from the eighth nerve which end in oval patches (maculae) of sensory epithelia.

The root of the acoustic nerve early separates into a dorsal and a ventral portion, the ganglions into dorso-posterior and ventro-anterior masses. From the latter emerges the anterior branch of the peripheral acoustic nerve; it supplies the sensory crests in the ampullae of the anterior and the horizontal semicircular canals, and the sensory maculae of the utricle and the saccule. The posterior branch, which issues from the dorso-posterior ganglion mass, likewise ends in four end organs: the sensory crest of the posterior ampulla, the macula of the lagena, the basilar papilla, and the amphibian papilla. On the basis of physiologic experiments it is assumed that the crests are receptor organs of the static sense and that the papillae are acoustic receptors. The functional significance of the three maculae is still in doubt.

b) The oval window is common to all amphibians and higher vertebrates. During the formation of the cartilaginous otic capsule, a membranous window remains in its lateral wall; later it becomes nearly closed by an independent cartilage, the operculum. In frogs, this lid first appears in larvae of stage 28 (hind legs with five toes, knees, not motile).

c) The perilymphatic duct arises in the space between the membranous labyrinth and the cartilaginous otic capsule, through involution of some of the filling mesenchyme. Starting at the oval window it circles the saccule dorso-caudally, contacts the amphibian papilla and turns toward the low median wall of the otic capsule. It then enters the cranial cavity through the perilymphatic foramen and expands into a perilymphatic sac. This represents the final condition attained by primitive perennibranchiates (Proteus, Necturus). In all amphibians with a basilar papilla, a further branch of the duct develops, and forms a second prolapse into the wall of the otic capsule. This may project toward the postotic foramen (e.g., in Rana catesbiana) or into a completely independent aperture in the floor of the otic capsule (cf. fig. 3). This opening, covered by an elastic membrane, is the round window. From it ascends a terminal limb of the perilymphatic duct, which ends at the basilar membrane. It may be pointed out that the perilymphatic foramen is the primitive forerunner of the cochlear aqueduct of birds and mammals.

d) The bronchial columella connects the round window with the bronchus and the lung sac of the same side of the body. When fully developed, as in larvae of stages 26 to 31, the columella is fastened to the center of the round window, pier-

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ces the dorsal aorta and then fastens to the bronchus. Here it divides into three portions. The innermost spreads as a fibrous layer over the upper surface of the bronchus and the larynx. The other two form a loop on the surface of the bronchus. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane. Within this frame the thin bronchial membrane bulges slightly toward the dorsal membrane.

The columella begins to differentiate immediately following the formation of the gill sacs (stage 25). It originates as a condensation of fibers from the mesodermal sheath, that surrounds the bronchial vestibule and fastens it to the base of the skull; i.e. from the suspensory of the seventh pharyngeal pouch. At stage 25 this bundle of fibers is still on the medial side of the dorsal aorta. Subsequently the aorta shifts inward and engulfs the columella, providing it with a tubular endothelial sheath. This arrangement is maintained from stage 26 until metamorphosis (stage 31).

The anatomic structure of the entire acoustic apparatus (fig. 3) indicates that *in frog larvae the lungs serve as ear drums*. Containing easily compressible air, they serve to magnify vibrations of the surrounding liquids. The passage of the columella through the dorsal aorta makes it free from the resistance of surrounding tissues which otherwise might have a dampening effect. The columella is a quite solid rod, composed of closely packed, stiff and tough fibers. Vibrations transmitted from the lung to the round window, will be imparted to the fluid that fills the perilymphatic duct. The oval window at the lateral end of the duct serves as a relief valve.

At the height of metamorphosis when the frogs get ready to climb on land, the part of the lumen of the dorsal aorta at the inner side of the columella quickly shrinks. At the transition from stage 31 to 32 the columella again lies at the medial side of the aorta and presently it begins to disintegrate (figs. 4, 5). The bronchi as well as the aortae shift away from the round windows which now border on the lymph sacs spreading under the base of the skull.

c) The *tympanic organ* consisting of auditory tube, tympanic cavity, and tympanic membrane is an acquisition effected within the order of frog-like amphibians. Litzelmann\(^7\) describes the rudimentary condition which it retains in the pelobatids. The organ arises through peculiar transformations from the dorsal part of the first pharyngeal pouch\(^8\). In frog embryos of stage 19 this pouch still shows the usual position between the first and the second visceral arches. But after hatching, during development of the small larval mouth (stages 21 to 25), its dorsal and ventral recesses become separated from each other through the development of a joint between the quadrate and the hyoid cartilages (fig. 1). In older larvae, these recesses project peripherally toward the larval skin; the middle part of the pouch is little developed and remains on the oral side of the quadrate and hyoid cartilages (fig. 1). The ventral pouch persists for a long time but produces no important derivatives. The dorsal recess becomes the basic rudiment of the tympanic organ. In larvae, its terminal knob is fastened to the subderma and the rostral quadrate, under the nose. The long and filiform connection with the pharynx runs along the quadrate, first outside of its muscle process (beneath the orbito-hyoid muscle) and then around its ventral surface (figs. 1, 2). Throughout the larval period the organ persists in this rudimentary condition. It becomes reactivated during metamorphosis. At the time when the arms emerge from the gill sacs, the head of *Rana clamitans* still shows an essentially larval structure; but laterally over the terminal knob of the pharyngeal recess one now finds a disc-shaped mesenchymal condensation, the rudiment of the tympanic ring and of the fibrous layer of the tympanic membrane (fig. 2). The figures 4 and 5 illustrate the rapid developments that occur during metamorphosis. The organ remains attached to the quadrate and therefore is carried caudally when the mouth assumes its adult size. The endodermal rudiment transforms into a funnel-shaped *tympanic cavity* and an *auditory tube*. The opening of the latter into the pharynx moves first to the front of the ear capsule, then ventrally under it. Concurrent with these developments, proceed growth and differentiation of the car-


tilaginous tympanic ring and the tympanic membrane. The latter consists of endodermal, mesodermal (fibrous) and epidermal layers. It forms a part of the body surface of the metamorphosed frog.

f) The tympanic columella is now generally believed to be an upper, i.e. cranial segment of the hyoid cartilage. This assumption is based on such observations as the proximity of the larval rudiment to the facial nerve (fig. 2) and of the finished ossicle to the horn of the hyoid cartilage (fig. 5). It begins to take shape late in the larval period, as a mesenchymal, T-shaped condensation in front of the operculum. It grows slowly toward the otic process and becomes cartilaginous. During metamorphosis, when the tympanic organ approaches, there forms a second mesenchymal rudiment, the plectrum. At stage 32 it lies within a still existing opening of the tympanic ring and above an indentation of the endodermal epithelium (fig. 4). It is attached to both the columella and the tympanic membrane. At the end of metamorphosis the columellar connection of tympanic membrane and operculum is complete (fig. 5). In species with shorter larval periods than Rana clamitans, this phase of the development of the ear is somewhat delayed.

Summary. The changes of the frog ear during metamorphosis may be summarized as follows. The tympanic organ and the tympanic columella replace functionally the lung sac and the bronchial columella as sound receiving and transmitting mechanical auxiliaries of the ear. The round and the oval windows exchange their relative roles, the former now becoming the relief valve of the perilymphatic duct. Except for the near complete ossification of the otic capsule, the central part of the ear remains unchanged. It must be concluded that for sound perception in the basilar and amphibian papillae, it is of little or no importance, whether the vibrations of the perilymphatic fluid start at the round or at the oval window. The larval condition resembles that of teleosts with swim bladders connected to the inner ears by means of Weber ossicles.

Discussion

Weber 10, as early as 1820 described a connection between the perilymphatic sinus and the swimbladder in certain taxonomic groups of teleosts, which are collected under the name of Ostariophysi. The connecting structure, consists of a series of three ossicles, said to be derived from the dorsal arches of the first, second and third vertebrae. On the basis of the morphologic evidence, Weber concluded that swimbladder, ossicles, and perilymphatic space serve as receptors and transmitters of acoustic vibrations. More than a century passed until some experimental evidence in support of this interpretation was supplied. In the freshwater fish Phoxinus laevis, Wolff (quoted by von Frisch 11) observed a 40 to 70 fold reduction of the hearing power after removal of the swimbladder.

Very little is known about the mechanics and physiology of hearing in amphibians. In view of their characteristic reactions to voices and to other sounds, it can not be doubted that frogs actually have a sense of hearing. This fact is also ascertained experimentally by Yerkes 12.—Even less than those of the adult frogs, were studied the acoustic capacities of the larval amphibians. Frog tadpoles exhibit flight reactions to sounds and to some mechanical vibrations; but to the author's knowledge neither the physical nor the physiologic aspects of this behaviour have been analyzed.

The assumption that the bronchial connection is, functionally, an accessory to the acoustic organ rests therefore entirely on the above reported morphologic observations and on comparisons with better known animal types. Considering that the lung sacs of the larvae seem to have an importance as hydrostatic organs, there appears also the possibility that the columella transmits changing pressure conditions to the entire ear and thus to the control organ of the statodynamic sense. Nevertheless it must be concluded that the whole accessory apparatus is more specifically related to the acoustic sense, for the following reasons.

1. The shift of the columella into the dorsal aorta is more important for the transmission of
sound vibrations of relatively high frequency than for that of the slowly changing tensions which must accompany hydrostatic pressure variations.

2. The columellar apparatus, by the medium of the perilymphatic duct, is attached to the saccule, not the utricle; and it is now generally conceded that the saccule is the receptor of acoustic impulses.

3. Special branches of the perilymphatic duct make contacts with the amphibian and the basilar papillae (fig. 3). Thus the construction of the perilymphatic system insures the conduction of vibrations of the round window to the specific sensory epithelia of the acoustic organ.

4. The presence, at the lateral end of the perilymphatic space, of an elastic relief membrane (the oval window) permitting the free play of vibrations, is indicative of the acoustic character of the organ (see below).

The contention of De Burlet\textsuperscript{13} that labyrinths without perilymphatic spaces are merely static organs, and that perilymphatic ducts and relief windows are indispensable parts of true ears, can no longer be maintained. Von Frisch and others (cf. von Frisch\textsuperscript{11}) have published evidence of hearing in some teleosts that do not belong to the Ostariophysi. Evidently, hearing is a very old and widely distributed capacity of vertebrates; but it gained greatly in sensitivity and possibly also in qualitative range through the evolution of the perilymphatic sound track. While in the primitive condition sound waves enter and pass the ear from all directions, in the ostariophyses, and the majority of anurans and higher tetrapods reception is specialized for vibrations between the round and the oval windows. This differentiation must favour the evolution of peripheral resonators, intensifiers and selectors. Du Bois-Reymond\textsuperscript{14} has pointed out that sound waves produce visible pulsating contractions and expansions of submerged air bubbles. In underwater hearing, the connection of the round window with the air-filled lung sacs therefore offers the possibility of an immense intensification of the peripheral impulses. On the other hand Wada\textsuperscript{15} with his exact studies, in adult frogs, of the tympanic vibrations in response to sound waves, has demonstrated the high responsiveness of the tympanic membrane to aerial sounds of a considerable range of wave lengths. Since aerial sound waves do not easily penetrate the solid body walls, the bronchial connection becomes practically worthless when the animal leaves the water. In adult urodeles and pelobatids the labyrinth becomes attached by various means to structures like shoulder girdles and jaws which, according to life habits may receive and conduct vibrations of different nature. The embryology and physiology of these primitive groups are not yet sufficiently known to furnish any clues about their possible importance for the elucidation of the pathway of the evolution of the tympanic organ. The morphology of the adult ears suggests, however, a progression leading from Pelobates over Bufo to Rana. Full efficiency in the reception of aerial sound waves seems to be attained only in the last named genus.

The similarity of construction of the bronchial and the tympanic accessory systems is remarkable. In both instances the peripheral end of the columella attaches excentrically, near the circumference of the vibrating membrane; it traverses an open space which in larvae is the dorsal aorta and in metamorphosed frogs a diverticle of the first pharyngeal pouch, the tympanic cavity. Centrally its attachment covers nearly the entire receiving window of the perilymphatic space. However, the mechanics of the bronchial and the tympanic membranes must rest on different principles. While the latter borders on air, with both its surfaces, the former lies between the air that fills the lumen of the lung and the coelomic fluid. Apparently it is the entire lung that vibrates and the bronchial membrane may mainly have cushioning and selecting functions.

At the time of metamorphosis the roles of the two perilymphatic windows are exchanged. Apparently the labyrinth organ is not affected by this reversal, and it does not show any structural remodeling. The oscillations of the perilymph must be the same, whether started at the round or at the oval window. The great importance of the relief window in permitting the free play of these oscillations may be recognized by the fact that its calcification, in man, leads to deafness,\textsuperscript{15}

\textsuperscript{15} Y. Wada, Beiträge zur vergleichenden Physiologie des Gehörorganes. Pflügers Arch. ges. Physiol. Menschen Tiere 202, 46—69 [1924].
and that hearing in such cases may be restored surgically by fenestration of the cochlear duct.

Embryologically, the two accessory systems are homologous in the sense that both are derived from pharyngeal pouches, the seventh and the first respectively. However the detail shows some significant differences. The bronchial columella arises from the suspensory ligaments of the (seventh) pharyngeal pouch while the tympanic columella is essentially a skeletal structure and develops as a part of the (second) visceral arch. The latter maintains permanently its position behind the first pouch and only its distal end lies in a fold of the endodermal wall of the tympanic cavity (figs. 4, 5).

From the viewpoint of constructional economy and the resultant quality of performance, the development of the frog ear during metamorphosis represents an astounding example of adaptation to changing environmental conditions.

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**NOTIZEN**

3-Oxy-kynurenin als $cn^+$-Gen-abhängiges Glied im intermediären Tryptophan-Stoffwechsel

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Frühere Untersuchungen¹ haben ergeben, daß die in Abhängigkeit von mehreren Genen verlaufende Bildung der Ommochrom-Pigmente bei Insekten sich in bezug auf die Wirkung der Gene $v^+$ und $cn^+$ (Drosophila) nach folgendem Reaktionsschema vollzieht:

$$
\begin{align*}
\text{Gene:} & \\
\text{Gen-Fermente:} & \\
\text{Substrate:} & \\
-I. \text{Tryptophan} & \rightarrow \hspace{2cm} II. \alpha-Oxy-tryptophan & \rightarrow \hspace{2cm} III. \text{Kynurenin} \\
& & \rightarrow \\
& & \rightarrow \\
& & \rightarrow \hspace{2cm} \text{cn}^+\text{-Stoff''} \rightarrow \text{Ommochrome}
\end{align*}
$$

Abb. 1.

Danach besteht die Aufgabe der Gene $v^+$ und $cn^+$ in der Bereitstellung spezifischer Fermente, die einzelne Schritte in der zum genabhängigen Außenmerkmal (Pigment) führenden Reaktionskette des Tryptophan-Stoffwechsels katalysieren.


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² R. Danneel, Biol. Zbl. 61, 388 [1941].