First single movement of turkey embryos

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Key words: embryonic occipital muscle, movement, notochord, floor plate, somites, biomechanics, live observation.

Summary. The purpose of the study was to characterize the first contraction of an isolated muscle in turkey embryo. The space of time of the contraction since beginning of incubation, topography, morphogenesis and histology of the concerned muscle and its mechanical counterpart are described.

Material and methods. From the 3rd day of incubation on until the 6th day the embryos were continuously watched through a cellophane window in the eggshell. The installing of the window followed a certain time schedule to reveal the influence of the experimental conditions. For histology the embryos were fixed in Bouin’s fluid, then completely cut in serial sections of 5 µm thickness and stained according to Masson-Goldner’s trichrome procedure plus resorcin-fuchsin. Wire frame 3D reconstructions were performed to reveal the topography of the region.

Results. A paired muscle 1 mm long and 0.1 mm broad, derived by fusion of the four occipital myomeres, is responsible for the first individual contraction. The contraction produces a stretching in the neck region. The muscle named M. occipitalis primordialis consists of four end-to-end connected groups of mononucleated muscle cells; insofar it looks like early muscle in fishes and amphibians. The muscle contains two types of cells according to the cell nuclei. The elastic rod-shaped notochord represents an endoskeleton. Immediately after contraction it brings the body of the embryo back into its former shape. In the neck region the diameter of the notochord is less and, therefore, the elasticity of notochord is higher than further caudal. The floor plate may prevent damage of the neural tube during excursion of the notochord. The floor plate is flanked by two floor plate posts, which have a filamentous content like the floor plate. Their function may be fastening of floor plate and protection of nerve tissue. The passive pulse movements in the occipital region for 2 days before first contraction are considered to be of importance in orientation and extent of the consecutive active reactions.

Conclusion. Vital observation accompanying serial section examination showed to be a suitable approach to biomechanical investigations in embryology. It allowed even after nearly 180 years of intensive morphological studies of avian embryos to find a new, up till now not described muscle.

Introduction

Genetic and molecular biological methods are nowadays preferred to describe and explain morphogenetic pattern and developmental mechanisms in embryology. A variety of experimental procedures are now available, for instance: genome alterations like insertion or knock-out of genes followed by studies of the mutants’ phenotype, immunohistological localization of transcription regulators which activate specific genes, isolation and culture of stem cells to study their potentiality in vivo and vitro, tissue transplantation or ablation in vivo, tissue reconstruction in vitro, and many more. This is repeatedly reviewed (1–7).

Human embryology profits from the fact that many aspects of embryology are comparable in all vertebrate classes. Avian and mammalian (amniotes) embryos are particular similar (5). But there are more perspectives to consider than molecular biology and genetics in embryology (8).

In every moment, an embryo has to preserve all its vital functions in addition to its task to follow a specific morphogenetic pattern and become mature. Mo-
ovement of cell organelles, cells, cell groups, parts of or whole organs, trunk, and extremities are obvious signs of vital functions, which have to be considered as an integral part of the developmental process. Embryonic movements directly accessible to an observer by looking through a window in the eggshell are very conspicuous, and since K. E. von Baer (9) first description carefully studied, filmed, and described (10–12). Three kinds of primary movements are distinguished: a) beating of the heart, b) passive oscillation of the embryo (embryokinesis), and c) active autonomous movements (13). A biomechanical analysis of these first embryonic movements with definition of active and passive components in kinesiological sense (14, 15) has not been done up until now. On the other hand, the early stages of myogenesis (16, 17) and its continuation in myotome formation are repeatedly reported (3, 18–22). However, where and how the first movements of the embryo appear cannot be derived from these reports. The notochord could be considered as the main passive counterpart of contractions. It is the stiff, elastic axial skeleton most prominent in lower vertebrates. Although it is present in avian and human embryos, a mechanical function of the notochord in these species is denied or not dealt with (23–28). In higher vertebrates, the notochord has only a very short lifespan (28, 29); cartilage and bones soon replace it. The specific morphology of the notochord is in accord with its proposed biomechanical function (26–28). The notochord has furthermore a very remarkable influence on the development of the muscle tissue (30–35). The connective tissue, which transfers the forces from muscles to the target tissue, should not be neglected in biomechanical consideration (36–40). In order to get more information upon the biomechanical situation, turkey embryos were watched until the appearance of all three kinds of movements. The specimens were fixed and cut in serial sections. 3D reconstructions from serial sections were prepared to assist morphological interpretation.

Material and methods
Fertilized turkey eggs, B.U.T. Big 6, were obtained from a commercial supplier and incubated at temperature of 38°C, humidity of 45%, with continuous ventilation, and turning every hour to an angle of 90°. For vital observations, eggs were windowed by sawing a 1 cm2 large opening in the top of the egg shell and covered by an autoclaved cellophane foil. A ZEISS stereo dissection microscope, which allowed magnification up to 4 times primarily, was used for the vital observation. A halogen light source with two fiber optic cables was taken to illuminate the embryo. After observation, the eggs were returned to the incubator to keep them under conditions as before, though turning was not possible anymore because of the window. Vital observations were performed from the 3rd to the 6th day of incubation. Twenty eggs were used according to the following schedule in order to find out the influence of experimental conditions: starting after 3 days of incubation, i.e. after 72 h, 5 eggs were windowed and the minutes of the developmental stage examined. The next 5 eggs were windowed 6 hours later and again after 6 hours, i.e. in total after 78 resp. 84 hours of incubation. The newcomers were compared with those already under observation. The last 5 eggs were opened 12 hours later, i.e. after 96 hours incubation. The observations were continued until the 6th day, i.e. 144 hours of incubation.

Another cohort of incubated eggs was used for histological studies. Result of the vital observations let us to concentrate the histological study on the 5th day. Embryos after 3, 4 and 6 days of incubation were also fixed. The embryos were fixed in situ by injection of Bouin’s fluid under the vitelline membrane resp. into the amnion cavity. After 2 hours the embryos were freed from the extraembryonic membranes under the stereomicroscope, than returned for 6 to 24 hours into the fixative and transferred into 80% ethanol. After dehydration the specimens were embedded in paraffin in the desired position. The embryos were sectioned completely with a Leica microtome in serial sections of 5 μm thickness. The series were stained according to Masson-Goldner’s trichrome procedure plus resorcin-fuchsin. Five horizontally sectioned series each containing about 1100 slices were available.

The most symmetric one regarding left-right correspondence was chosen for 3D reconstruction. A total of 250 slices belonged to the region of interest between the top of the metencephalon and the upper border of the heart. From these, 40 slices in regular intervals were chosen to perform the drawings for the reconstruction. The drawings were made by a ZEISS microscope-drawing device with primarily 2.5 objective, finally 30 times magnification. All outlines and the inner structures of the embryos were drawn as complete and carefully as possible from the slices. Small structures could be diagnosed in higher magnifications and were then labeled in the drawing. The drawings were then piled up on a light screen according to best visual fit and supplied with a joint reference coordinate system. According to this reference system the drawings were orientated on a scanner and the structures of interest digitized with Microsoft Coral Draw (Version 8).
digitized data were transferred to Microsoft Excel, and along with a calibration bar converted into x- and y-coordinates of the structures. The z-coordinate was gained from slice thickness 5 μm. The zero point was set at will, so that the data are positive. To avoid hiding of distant structures by those nearby wire views were applied. Because mainly tubular structures are regarded, the display of their centers is sufficient. To cope with artefacts from distortion of slices, imprecision of drawings, and adjusting of the reference system, interpolation procedures become necessary.

Interpolation was done by replacing each coordinate by the mean of its 6 neighbors. This was done twice, i.e. first in one and then in the other direction because of the peculiarities at the ends. The 3D manipulations were done by matrix multiplication for translation, x-, y-, and z-rotation, their combinations, and furthermore for the perspective view for the right and left eye in Microsoft Excel as described earlier (41). Without suitable eye glasses it is difficult for an inexperienced observer to get the perspective view from separate right and left images, therefore, only one image is given here. Labeling of the structures seemed to be sufficient to describe the situation. The diagrams can easily be plotted by the diagram-assistant of Excel, but for detailed labeling the diagrams had to be returned to Corel Draw to give them their final appearance.

Photos from the slices were taken by ZEISS Axiophot. All statements regarding the size of structures are based on the direct comparison in the ZEISS drawing device with an Olympus objective micrometer 0.01 mm.

Results

Live observation of the embryos was done through a cellophane window in the eggshell using a stereomicroscope:

72 h (about): Five eggs are opened. All embryos show continuous beating of the heart. The frequency of beating is between 40 and 100 beats per min and depends on the temperature. The heads begin to turn, resp. are already turned to the right side. The cranial flexure begins to occur. The trunks are lying completely stretched on the surface of the yolk sac. A cervical flexure does not exist. The blood stream, offering the observer optimum orientation because of the red color, flows through the first (mandibular) and second (hyoid) aortic arches and enters the embryonic vascular system. The pulsation of the heart is transmitted through the arches to the dorsal aorta, were the arches unite. The blood flows downward to the trunk through the dorsal aorta, which subsequently divides into a right and left part. The blood flows upward from the dorsal aorta through the internal carotid artery to the head. The pulsating blood stream forces the dorsal aorta to a continuous oscillation. The cardinal veins return the blood to the heart.

78 h (about): The five new embryos show nearly the same stage of development as those already studied. Therefore, the description of the developmental stage is given for all together. The small individual variations are not considered in this report. The head is now rotated about 90°, while the trunk is still prone on the surface of the yolk sac. Therefore, the axis of the embryo is twisted in the intermediate region. The cervical flexure appears at the junction of head and trunk. The heads sink a little bit into the yolk sac, and the head-fold of the amnion appears. The heart generates repulsion of the heart ventricle onto the wall of the yolk sac near its stalk, and propulsion via the blood stream onto the aortic arches and further to the dorsal aorta. The pulsation is directed according to a line starting from the heart, passing the two aortic arches ending in the middle of the cervical flexure.

84 h (about): No great differences in development among the previous 10 specimens and the five undisturbed incubated and moved eggs are evident. Head and heart have considerably enlarged and are now completely positioned below the level of the pellucid area. Two or three aortic arches are very prominent. The head is pushed dorsally with every heartbeat. The dorsal aorta regularly sends off short branches, the segmental arteries. Because of this the somites are now easy visible. The circular vessel of the right eye appears.

96 h (about): The development of the embryos from the newly opened five eggs fits well with the stages of the others. The cervical flexure is now much more prominent. The angle between hindbrain and trunk approaches a right angle. The cranial part of the embryo is now loosely hanging in the amnion cavity and oscillates with every heartbeat. Each beat pushes the neck region somewhat dorsally. It is obvious that the cervical flexure is opened a bit with each pulse, and the hindbrain stretched. The hindgut forms a bay and also starts to turn but is still motionless.

102 h (about): The head floating in the amnion cavity is rhythmically moved by the beating heart. The cervical flexure is pushed dorsally stretching the head with each beat. The amnion has reached the umbilical region. The tip of the tail begins to be directed forward. Curving of the tail and increasing of the cervical flexure account for the decreased length of the embryo.

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No allantois is visible. The beginning of outgrowth of the right fore limb bud and hind limb bud is indicated by localized thickenings of the body wall.

116 h (about): First single spontaneous muscle contraction is seen. The contraction originates in the occipital region of the embryo. The contraction can be watched from above, if the embryo holds its normal position. The movement lifts the hindbrain a bit relative to the trunk. Primarily it is a stretch movement, which is immediately followed by return to the initial position. The contraction occurs on right and left sides more or less simultaneously. A simple nod movement results. The location of the contraction lies cranially from the first visible (cervical) somite. A special structure is not discernible, not even with the high resolution of a stereomicroscope. This region is easy to localize. It is almost encircled by the occipital vessels of the anterior cardinal vein, and can be found in prolongation of the direction of the undermost aortic arches into the occipital region. It should be mentioned that this line also points to the midst of the cervical flexure. In two specimens the oblique orientation of the embryo in the egg impedes watching the occipital region. In all other cases a contraction could be observed. At the beginning the intervals between spontaneous contractions varied considerably within one embryo as well as among the embryos. The interval lasts most often a minute, but sometimes one has to wait 5 minutes, or longer. Contraction can be evoked by a short shake of the egg. The embryo is fixed only by the vessels at the umbilical cord to the yolk sac. The whole embryo including the tail is floating in the amniotic cavity. Some hours later short episodes of whole body movement caused by contraction of the amnion (embryokinesis) also happened for the first time. Allantoic vesicles had appeared.

144 h (about): The whole embryo presents whole body and single trunk movements. Amniotic contractions appear regularly and later on continuously. At the beginning the whole body is drawn caudally, kept there a moment, and then released again. The spontaneous occipital contractions are mostly synchronous, but sometimes appear also asynchronous. Asynchronous contractions produce turning of the head, but the head immediately returns back. Correspondence between occipital contractions and whole embryo movement could not be registered. Some somites between the limb buds also started to contract. If these contractions are not simultaneous on left and right side, the trunk twists, but it always returns to its former position. In this way movements of the embryo become three-dimensional.

**Microscopic observations**

The occipital region influenced by permanent pulse beat and locality of the first spontaneous contraction is now thoroughly reinvestigated. Five days after incubation a very loose mesenchymal tissue occupies the retro- and suprapharyngeal space (Fig. 1).

![Fig. 1. Horizontal section through the retropharyngeal space of a turkey embryo after 5 days of incubation](image)

Ph = pharynx; M = loose mesenchymal tissue; rdA = dorsal part of the right aortic arch; ldA = dorsal part of the left aortic arch; C = anterior cardinal vein; Not = notochord; T = neural tube.

This axial mesenchyme appears loose because the mesenchymal cells keep distance from each other. Cells of different kind are found. Some cells have more round nucleus with one or two prominent chromat bodies. Very thin cytoplasmic processes extend from the cell body. Other cells have elongated or triangular nuclei, broader and longer processes. The loose axial mesenchyme ends caudally at the fusion of the aortic arches giving rise to the impaired dorsal aorta. The loose axial mesenchyme is bordered by the dorsal and apical epithelial wall of the pharynx, by the aortic arches, by the anterior cardinal veins, auditory vesicle, scleromatal cells, notochord, floor plate, and floor plate posts (see later) (Fig. 1). More cranially, where the notochord separates from the floor plate the very loose tissue takes place between these structures. Small vessels spread into this tissue (Fig. 2).

In the paraxial mesenchyme of the myelencephalon dorsal from the notochord small bundles of nerve fibers leave the encephalic wall and enter the hypoglossal nerve. The nerve first adheres to the neural tube, then gets in more distance of it, and splits. Lateral from the nerve in horizontal cranial-caudal series the somite cervical 1, still with a somitocoeil, and the last occipital somite derivate appear. It is called somite.
derivate, because in this stage only the myotome is left and this has been differentiated to a small muscle. This is also true for the other occipital myotomes, which appear in the consecutive sections.

**Musculus occipitalis primordialis**

Characteristic features of the occipital myotomes on the 5th day of incubation are the lack of a dermatal epithelial layer and a lack of the cleft between (Fig. 3, 4 and 5). Because of this they are easy to recognize also in low magnification (Fig. 3). The four occipital myotomes join to a small muscle, which contains two types of cell nuclei. Type A takes position in the center of the muscle segment (Fig. 4). The nuclei are larger than the others; possess elongated shape, pale nucleoplasm, a marked nuclear membrane, and one to two prominent round chromatin bodies. Type B is found near the ends of the muscle segment and insofar on both sides of the type A group. The nuclei of type B are smaller, more spherical and the interior looks more similar to the surrounding mesenchymal cells. It is not possible to assign definitely the bundles of intracellular myofilaments to one or the other or to both cell types.

According to the four-shared occipital myomeres the muscle has 4 segments.

The most cranial segment is very rudimentary and appears only in some neighboring sections, covering wide perhaps 30 μm, and the 4th segment has a cleft to cervical 1. In addition, it has an epithelial, dermatomal cap near this cleft (Fig. 3). The muscle has a total length of 1.0 mm with the individual length from about 1st segment to 4th segment 0.5; 2; 3; 4.5 mm. Its width is about 100 μm. The four parts are not only connected end-to-end, but at these junctions they have also connections to the surrounding mesenchyme, the sclerotome (Fig. 5). The embryonic occipital muscle lies mainly dorsal of the notochord. Only the anterior tip of the muscle was found in the same horizontal section as the notochord.

**Notochord**

In the suprathyngelus space the notochord is, shortly before it ends, isolated in the loose axial mesenchyme (Fig. 2). It is separated from the rhombencephalon footplate by 0.16 mm. Vessels are found in
the mesenchyme between these two structures. From the level of confluence of the aortic arches to build the dorsal aorta on, the notochord is quite near to the neural tube, separated only by two cell layers. In its supra- and retropharyngeal part the notochord has a diameter from 40 μm to 80 μm. The two outer borders with a light space in between are clearly seen (Fig. 6 and 7). The vacuoles are numerous and in horizontal sections, i.e. when cut longitudinally, possess a transverse, right to left, elongated form (Fig. 6 and 7A). At the level of the aortic confluence, the notochord becomes broader and in transverse sections the vacuoles appear with round profile (Fig. 7B). In the middle of the embryo’s body the diameter of the notochord is about 110 μm and filled with several round vacuoles. In the caudal flexure, the notochord is found again in stretched form and horizontal position as cranial. The vacuoles are elongated in

Fig. 5. Junction between segment 3 and 4 are shown
S = space between muscle and adjacent tissue; Junc = junction between segments of the muscle as well as with the sclerotome.

Fig. 6. Horizontal section at the level of confluence of the aortic arches forming the dorsal aorta
Not = notochord; F = floor plate; P-F post of the floor plate; V = vessels; OM = primordial occipital muscle; Neural tube = neural lumen and neural wall.

Fig. 7. Sections through the notochord in the same magnification, cut in the suprapharyngeal region longitudinally (A) and transversally (B) and in the returned tail-end (C)
In A = notochord with right-left elongated vacuoles; in B = notochord with a diameter a diameter of 85 μm; in C = notochord with a diameter a diameter of 50 μm.
right-left direction as in the cranial part. The diameter is reduced from 110 μm to 80 μm. In the returned, tail part notochord has a diameter of 50 μm and less and contains no vacuoles (Fig. 7C).

**Floor plate**

The floor plate is a prominent structure in turkey embryos incubated for 5 days (Fig. 2 and 6). It has an inner filamentous layer, a cell nucleus layer, and an outer filamentous layer (Fig. 6). The inner layer, at the lumen of neural tube, is built of densely packed, straight radial filaments; the next layer is composed of small spherical cell nuclei, and the outer layer again shows filaments but arranged transversally to the neural tube and notochord, and they are more loosely packed than in the inner layer. The spherical nuclei of the middle layer possess a central positioned spherical nucleolus and differ insofar from the nuclei of the other cell of the brain wall. Mitoses appear on the inner side of the nuclear layer. The floor plate is an especially prominent structure in the mesencephalon and metencephalon at the observed stage. Here it is about 100 μm broad at the inner border and 250 μm at the outer border. It becomes already smaller in the myelencephalon, namely 40 μm inner distance resp. 150 μm outer distance, while at the dorsal aortic confluence the values are 20 μm resp. 100 μm. The footplate measures 20 μm at the inner and 80 μm at the outer border at the level of the upper limb. These sizes remain further caudal until the returned part, the tail part, where the floor plate finally disappears.

**Floor plate post**

On both sides of the floor plate two remarkable structures appear, which contain filaments similar to the outer layer of the floor plate but with the filaments orientated at a right angle. They are positioned sagittally (Fig. 6). In literature no special attention is given to them, the structures are labeled ventral column of white matter (42). Because of its peculiar structure, relationship in internal structure to the outer layer of the floor plate, and its peculiar size changes, it is named here “post of the floor plate” to separate it from the remaining marginal layer of the ventral column, which looks different. The two posts of the floor plate may surpass the floor plate to ventral in the myelencephalon region (Fig. 6). The orientation of the filaments in floor plate is vertical; the filaments in the post are orientated sagittally. On both sides on the surface of the floor plate posts vessel frequency mark their borders (Fig. 6). The posts of floor plate are prominent, where the notochord is small and where loose mesenchymal tissue appears in front.

**Fig. 8. 3D reconstruction of the ventral aorta beginning in the heart (h) continuing with the aortic vessels of the 3rd and 4th visceral arches on the left (3l and 4l) and right side (3r and 4r) and further continuation with the dorsal aorta (Ad) and the internal carotid on the right side (ir) and on the left side (il)**

Notochord (n) is also visualized. Arrows point into the direction of blood flow, which corresponds to the direction of the main blood pulse excursion. View (A) is from ventral, left, a little bit from below to show that the main convex indentations of the aortic arches fit into the concave indentation of the notochord. The view (B) from ventral, above shows the considerable excursion of the blood flow first to lateral, then return to medial, and this in addition to the turn around of the main blood stream from cranial to caudal region.

**Reconstruction (Fig. 8)**

To assess the possible mechanical influence of the heart activity by pulsed blood stream on structures of the embryo in the occipital region, the aortic vessels were three-dimensionally reconstructed. The first view (Fig. 8A) is from left/ventral to right/dorsal. At the stage considered the aortic arches through the 3rd and
4th arches are the main output vessels of the heart. The thrown out blood is divided immediately after leaving the truncus arteriosus into a lower right and left, and soon again into an upper right and left stream. All four aortic arches impede the blood stream from going strait ahead. The arches force the blood stream to turn at the beginning at an angle of about 60°, then by continually bending go to the left resp. to the right and dorsally. Finally, the blood flows nearly opposed to its original direction. Dividing and turning of the blood stream result in a buffer function of the aortic system. Pressure, which the blood stream brought upon the vessel wall, is not absorbed completely in the wall, but transferred to the tissue behind. The aortic arches pulsate active as could be seen in vivo because of the red color of the blood, while the tissue behind could not be evaluated. Only the notochord, not other structures, is included in addition in the diagram of the aortic vessels to avoid overcharging the figure. It becomes apparent that the bending of the aortic arches corresponds to the cervical bending of the notochord. The view in reconstruction (Fig. 8B) is from ventral/cranial to dorsal/caudal. The notochord lies in the middle, and its bending is again obvious. The space between the aortic arches and the notochord is the region where the mechanical load of pulsation culminates. This region is filled by loose mesenchymal tissue as seen in Fig. 1 (M). This occipital region is bordered, as already mentioned, ventral by the dorsal and apical epithelia of the pharynx (Fig. 1) and dorsal by notochord, floor plate, and floor plate posts in front of the nerve tissue and m. occipitalis primordialis with adhering sclerome on both sides.

Discussion

The purpose of this report is to refer to structures and conditions responsible for the first single movement of the embryo. All early embryonic movements have two phases as became immediately apparent when watching the living embryo: dislocation and restitution. This is true for the continuous beating of the heart, the autonomous contractions, and the amniotic whole embryonic movements. The first single spontaneous muscle contraction always appeared at the same place in the occipital region. Bearing probable influence of the experimental condition in mind, the eggs were not windowed for observation at the same time. Some embryos were kept for longer periods in undisturbed incubation, but all showed the phenomenon with common variance at the same time. After five days of incubation, the first single spontaneous muscle contraction can always be seen in turkey embryos. In histological preparations the small occipital muscle derived by fusion from the four occipital myotomes is the only candidate that can produce the first contraction, and m. occipitalis primordialis in reminiscence of the “primordial cranium” will be considered as an adequate name. This paired embryonic occipital muscle without dermatomal cap looks similar in appearance to fishes’ and amphibians’ early muscles (43–46) and different from the remaining dermomyotome of hen and turkey at this stage mainly because of the absence of the intersomitic clefts (18, 19). In fishes and amphibians mononuclear muscle cells cover the whole length of the myotome. Multinuclear myotubes are formed in later stages (44). The cell nucleus of the uninnucleated muscle cells in fishes’ and amphibians’ myotomes (43–46) looks like the cell nucleus of type A (Fig. 5) here. These cell nuclei take the central position in the myotome here and there. Therefore, the cells with type A nucleus should be the main contractile cells, namely uninnucleated muscle cells.

Cells owning nuclei type B could be supporting cells or myoblasts, becoming type A nuclei cells. O’Rahilly & Müller (47, 56) have described the 4 occipital somites in human embryos and also observed their fusion, but did not present more histological details.

The left and right primordial occipital muscles are arranged longitudinally to the body axis, and are found dorsal of the notochord. This position is in accordance with the observed effect of their contraction in living eggs, namely opening of the cervical flexure by stretching, thereby drawing the head backward. Whether the muscle is already supplied by fibers from the hypoglossal nerve cannot be decided. Fibers of the hypoglossal nerve were found near the muscle, but not in direct contact. Nerve supply does not seem to be essential for early embryonic muscle activity because amniotic contractions happen also without nerves (10, 12).

The notochord is well known in lower and higher vertebrates, but its function in higher vertebrates is either denied or not given special consideration (23–28). In a biomechanical sense the notochord must be considered as the main structure to preserve the bodyform in embryos of higher and lower vertebrates. Earlier studies considered the morphological and histochemical changes of the notochord according to the age of the embryo (28). Our serial sections discovered remarkable regional differences in size and structure.
of the notochord in five days old turkey embryos. The
diameter of the notochord increases from cranial to
caudal nearly twofold, and then it is reduced again. In
relation to outside (vitelline vessels) the notochord
has a cranial horizontal, middle longitudinal, caudal
horizontal and short tail longitudinal part.

Considering the notochord vacuoles also in sec-
tions of different directions from other series, the
vacuoles must have the form of flat ellipsoids, which
are longitudinal, staggered all over in the notochord
in a similar way: “pile-of-coins” arrangement of Ju-
rand (28). Bending of the notochord would force
the vacuoles to increase their surface area, and that
would put up mechanical resistance, explaining its elasticity
(14, 15). The morphological vacuolated aspect is equal
in cranial and caudal parts; therefore, the basic internal
elastic properties may be the same. Taking the diam-
eter into account the notochord should be more elastic
in its cranial than in the main part of the body. It is
obvious, that the occipital muscle can stretch the head
only, if the resistance against this movement can be
overcome.

The live observed movement requires a mechanical
swing balance (48) between occipital muscle force
and local viscoelastic resistance mainly as response
of the notochord. How may such balance arise? The
live observation revealed that the occipital region is
under continuous mechanical stress of the beating
heart since the second day of incubation. Notwith-
dstanding the buffer system of the aortic arches, the pulse
beating reaches the occipital region visibly. The heart
supplies not only the small embryo itself, but in addi-
tion transports the blood to all the faraway capillaries
of the yolk sac and returns the blood. All blood has to
pass the aortic arches. The pulse is forwarded strongly
into the occipital region. The mechanical stimulus hits
the cervical flexure, the notochord, and the primordial
occipital muscle directly as could be derived from 3D
reconstructions. The resulting oscillating tension of
the tissue may be responsible for suitable muscle force
as well as for the corresponding notochord elasticity.
Tissue tension, in this case oscillating tension, may
be one of the local factors, which support genetic
regulation mechanism in myogenesis (3, 17, 49) as
well as in sclerome formation (22, 50). Ventral axial
tissue, notochord and floor plate induce sclerome
(51) via secretion of the protein Sonic hedgehog (52),
whereas ventral and dorsal structures are critical for
myotome formation (30, 32, 33, 53, 54). Interrelation
of secretion and reception of induction and growth
factors on one side and lively mechanical interplay
on the other have to be elaborated in future (48, 49).

The notochord looks like a u-shaped spring fading
in softened ends, responsible for keeping the embry-
onic body in its shape. The elasticity of the notochord
as a whole should be of substantial importance for
restoring the embryonic body shape, when the am-
nion sac has compressed the embryo and drawn to
the umbilical region. The floor plate at the observed
stage is a prominent and remarkable structure. The
outer filaments are arranged transversely to the no-
tochords axis. If the notochord is a moving viscoelastic
rod, then the floor plate is a cushion that protects the
neural tube from damage. The compactness and trans-
verse arrangement of the filaments would correspond
to this function. The notochord induces floor plate
(55). The two posts of the floor plate would not be
able to buffer the notochord, but perhaps they keep
the floor plate in its place. If this hypothesis is cor-
correct, the notochord and floor plate not only secrete
sonic hedgehog together (30, 33, 52), but they also
cooperate in a mechanical task.

**Conclusion**

The present study describes the location and struc-
ture of a new discovered muscle, *m. occipitalis pri-
mordialis*, in turkey embryos. The muscle derives from
the four occipital somites. It consists of serial con-
ected uninucleated muscle cells. *In vivo* observation
revealed that it is the first skeletal muscle, which con-
tracts. The contraction first time appears after 116
hours of incubation. The contraction, which results
in a stretching in the neck region, is immediately fol-
lowed by restoring movement. Notochord, mainly
responsible for this restitution, differs in diameter and
vacuolization from occipital to caudal region. For the
last 48 hours the region of the first spontaneous move-
ment was under remarkable mechanical influence of
the beating heart similar oriented in space as the con-
traction and restoring movement happened. It is con-
cluded that the mechanical balance between contraction
and restoring movement results from these heart
caused peculiar features of the tissue in the occipital
region.

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Pirmieji pavieniai kalakutų embrionų judesiai

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Raktažodžiai: embrioninis okcipitalinis raumuo, judesys, chorda, dugno plokštės stulpelis, somitai, in vivo stebėjimai.


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References
17. Arnold HH, Braun T. Genetics of muscle determination and
42. Lillie FR. The development of the chick. 2nd ed. New York; Henry Holt and Company; 1930.