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HISTORICAL SECTION

The Prenatal Development of the Human Orbit

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ABSTRACT During the 1970s, as part of his work for a doctor's thesis in which he described the development of the human orbit in great detail, the first author established the largest anatomical collection of embryonic and fetal orbits ever. Unfortunately, he died before the thesis could be finished. The thousands of sections have now been scanned at high resolution and made publicly available on the Internet at www.visible-orbit.org; 3-D reconstruction software is being developed. The Discussion and part of the 'Methods' section of this thesis are published in translation in this article. The conclusions of the first author at the time read as follows: (1) initially, the developing orbit is vaguely indicated by condensations in the mesenchymal connective tissue area; (2) in this connective tissue area, chondral, osseous and muscular structures develop and grow until, in the fully developed stage, the orbital content is surrounded by bony surfaces with a thin layer of connective tissue as periosteum, and by a muscle fragment; (3) the embryonic and early fetal phase, during which one can only speak of a 'regio orbitalis,' is followed by a period in which we can speak of a primordial orbit; (4) the phase of the primordial orbit extends until after birth; (5) the surface area of all orbital walls increases more or less linearly; (6) the 'musculus orbitalis Mülleri' occupies a special place in the orbital wall; (7) the so-called 'regio craniolateralis' is the primordium, which, in the fully developed stage, is occupied by the thick intersection of the frontolateral and the horizontal part of the frontal bone; (8) in the frontal plane, the shape of the primordial orbit, as well as that of the fully developed orbit, is more or less round; (9) the prenatal development of an eye socket is a complex event, characterized by changes in composition, shape and size of the orbital wall; and (10) the orbit can only be denoted by the term "eye socket" when it is fully developed. At the end of the thesis, he also presented the following postulates: (1) in the prenatal orbit, the development of the so-called 'periorbita' is at the forefront; (2) the mutual rotation of the orbital axes and the frontalization of the eyes from approx. 180° in the early prenatal stages to approx. 50° in adulthood do not seem to be caused by mechanical influences of the surrounding tissue; (3) the pterygopalatine fossa and the 'cavum cerebri' are not part of the orbit at any developmental stage; (4) in the prenatal skull, the inferior nasal concha, which forms part of the maxilla in the fully developed skull, is

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part of the 'capsula nasalis'; and (5) in order to achieve normal development of the eye socket in microphthalmus and anophthalmus, the normal orbital content should be restored.

METHODS AND MATERIALS

The study was carried out on the orbits of embryos and fetuses obtained from the Anatomical-Embryological Laboratory, the Department of Obstetrics and Gynecology and the Laboratory of Pathological Anatomy of the University of Amsterdam. In addition, we studied the orbits of a child that died nine days after birth due to intracranial hemorrhage as the result of mutilation. From this material, we selected embryos and fetuses that showed no external abnormalities. Material that was macerated, damaged or malformed was put to one side. The embryos and fetuses were fixed in 4% aqueous formaldehyde for two to three weeks, depending on their size.

Following fixation, the heads of fetuses larger than 100 mm were decalcified in 5% nitric acid for 24–72 hours, during which the process of decalcification was followed by means of X-rays. In order to eliminate the acid, they were then rinsed in running water for the same length of time as taken for the decalcification.

Next, they were dehydrated in ethanol of increasing concentration: 50%, 70%, 80%, 90%, 96% and 100%, two days in each concentration. Prior to embedding, the preparation was placed in a mixture of ethanol and ether (1:1) for eight days. Embedding then took place according to the following schedule:

−10 days in 5% Low Viscosity Nitrocellulose (LVN) Gurr in ethanol-ether (1:1)
−20 days in 10% Low Viscosity Nitrocellulose (LVN) Gurr in ethanol-ether (1:1)
−30 days in 20% Low Viscosity Nitrocellulose (LVN) Gurr in ethanol-ether (1:1)

This resulted in good penetration of LVN into the preparation. Next, the object was placed in 20% LVN for 24 hours in a glass vessel with a cover that did not completely close so that the LVN would become more concentrated. In order to allow the LVN to set, a layer of chloroform was then spread over the LVN. This setting

TABLE 1   Preparations of embryonic and fetal orbits selected for this study

Str. 74161, embryo, stage 16
Str. 73341, embryo, stage 22
Str. 73343, fetus, 42 mm crown-breech length (CBL)
Str. 73349, fetus, 76 mm CBL
Str. 73345, fetus, 79 mm CBL
Str. 75094, fetus, 113 mm CBL
Str. 74031, fetus, 144 mm CBL
Str. 74030, fetus, 150 mm CBL
Str. 74029, fetus, 217 mm CBL
Str. 77063, neonate

phase lasted up to the moment that the mild turbidity of the LVN that is caused by this treatment had reached the bottom of the glass vessel (24–48 hours). The block of LVN was then removed from the vessel, roughly shaped, allowed to set further and kept in 70% ethanol. The preparations that were selected in this way are listed in Table 1.

When measuring the length of an embryo or fetus, one can choose between the crown-foot length (CFL) and the crown-breech length (CBL). The latter measurement was used here because the legs of a fetus are bent and can only be stretched out by force after fixation. The crown could be found on a photograph of a lateral view of the fetus by drawing a line (a) between the back of the head (touching it at point (o)) and the shoulders (touching at point (s)) and then, at a point (d), drawing a line (b) perpendicular to it that touches the highest point on the head. This point was taken as the crown.

As the breech, we took a point that is found by drawing a circle with the greater tubercle of the humerus at its center, the circumference of which touches the caudal end of the trunk. This point of contact was taken as the breech.

The measurement was taken from point (d) on line (a), the head-shoulder line, past the contact point (o) until the contact point (s) and then along the back as far as point (h), which was already taken as the breech. Although a back that is bent is a bit longer than an outstretched back, this method was used in the study to determine the crown-breech length or "sitting height."

DISCUSSION
What is the Orbit?

One can only distinguish the orbit after pieces of bone and cartilage have been linked together by

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connective tissue that has differentiated into periosteum and perichondrium; this connective tissue, moreover, differs from the connective tissue of the primordial orbital content. In our series, this is not yet the case at the stage of 42 mm crown-breech length (CBL) but only at the stage of 76 mm CBL. One can speak of a primordial orbit from a moment between the two stages mentioned. At an earlier stage, we use the term 'regio orbitalis,' as long as there are no pieces of cartilage or bone connected by specific connective tissue around the primordium of the eye.

The Limitations of the Field of Study

In my research on the developmental history of the orbit, I have limited myself to the development of the bony fragments that contribute to the orbit. After all, the orbit is an important part of the skull, if only because seven of the twelve cranial bones are part of the eye socket.

Choosing the Material

Because collections of the immediately successive stages of human embryos and fetuses are scarce, collecting normal specimens was a prerequisite. Fetuses older than 26 weeks can only be obtained with the consent of the parents and the legal authorities. Only fetuses between approximately 20 and 26 weeks of age are widely available. For our study, we only had a limited number of embryos and fetuses at our disposal. The abnormal specimens were excluded on the basis of external macroscopic criteria and internal microscopic aspects. It was, unquestionably, difficult to distinguish variations within normal limits from the abnormal due to a lack of literature on orbital development. While studying orbital structures in a larger number of specimens than used for this thesis, we could, however, make a selection on the basis of a comparison of the morphological characteristics.

The Connective Tissue Components

It would be possible to devote a separate study to the development of each of the bony fragments that participate in the orbit and subsequently join them together into one orbit for each developmental stage. However, one would then be disregarding the continuity between the connective tissue areas in which the pieces of cartilage and bone extend themselves. The connective tissue is, after all, one continuous whole, in which one can-

not precisely determine which area belongs to which individual bone fragment. Thus, the question which area of the lateral connective tissue sheet belongs to the primordium of the zygomatic bone and which to the primordium of the frontal bone cannot be answered precisely. The primordium of the bony fragment consists initially of only locally interconnected bone spokes in type 6 connective tissue. There is also an extensive area of connective tissue on the orbital side of the bone fragments and, especially, in the primordial apex. The primordial orbit thus consists to a large extent of connective tissue: in, between and against the orbital bone fragments. The areas of connective tissue on the orbital side of the bone fragments can be called the primordial periorbita.

In this research, the connective tissue has been divided into six types. This distinction is based on the differences in the proportion of fibers, basic substance and tissue fluid in the extracellular matrix (Junquiera et al., 1984). The six types of connective tissue were distinguished on the basis of their fiber and cell structure. We did not make a finer distinction than the one based on simple light-microscopic criteria, since this study is limited to the microscopic observation of stages in the development of bone parts.

In this connection, we would like to point to the long history of very wide-meshed connective tissue in the craniolateral region, the superior orbital fissure and the optic canal. In this regard, it should also be noted that during the first half of the embryonic period, the primordial orbit consists of condensations of young connective tissue—mesenchyme—around the eye socket. We therefore call this the 'regio orbitalis.'

Development in General

A multicellular organism starts its development as a fertilized egg cell. In this development, the following stages can be distinguished:

a) growth
b) cell multiplication
c) morphogenesis
d) histogenesis, and
e) integration.

ad a. Growth can be defined as an increase in tissue mass and is caused by an increase in cell volume and cell multiplication.
ad b. Cell multiplication is caused by cell division.

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ad c. Morphogenesis refers to changes in the form of the organism or part of it. Changes in form are caused by the dissimilar growth rate of various structures mutually and each kind of tissue separately.

ad d. Histogenesis literally means: the formation of tissue. Tissues, composed of cells, similar or dissimilar in a single relationship.

ad e. Integration means becoming unified into a single entity.

Artefacts due to Fixation and Dehydration

When determining the length of embryos and fetuses, tissue changes as a result of dehydration and fixation with formalin and alcohol should be taken into account, as well as the deformation caused by making slices.

Scammon and Calkins (1929) did not always find the same degree of shrivelling in formalin-fixed fetuses and even, in some dimensions, an increase in size. They found "a very small decrease in the crown-rump length or sitting height, and the departure of the di-parietal diameter from the dimension as observed in the fresh material was a negative value, in contrast to the positive value as found by Schultz (1919). Our specimens showed an average decrease of 1 per cent while Schultz found an average increase of 0.88 per cent."

We have found no data in the literature on the effects of formalin and alcohol on various tissues. It is shown clearly by our observations that the cerebrum and the eye change in shape, most likely as a result of withdrawal of moisture from their cavities. It could also be imagined that wide-meshed connective tissue will shrivel more than condensed connective tissue because of their varying moisture content, so that cartilage might shrivel more than bone. An indication of such changes in shape of various tissues is given by the research results of Scammon and Calkins (1929). They found changes in the various dimensions of the skull. Table 5 of their publication shows that some dimensions decreased by 1% while others increased by 2.5%. We can therefore conclude that the skull changes in shape. Their measurements were average values, determined in 10 fetuses without mention of their size. The authors also examined the effect of formalin injections on the dimensions of the fetus. Here they indicated that they used 26 fetuses with a total body length varying from 36.8 cm (approximately 29 weeks old) to 54.2 cm (neonates). These injections resulted in an increase in nearly all measurements. It should be noted that the amount of formalin injected was 30% (!) of the total body weight of the fetuses.

Our material was only fixed in formalin by immersion, not perfused or injected with it, and we can conclude that the shape may have varied to a certain extent but that the changes were slight. Therefore, we have made no corrections to our calculations of bone surface areas. Apart from that, in the literature available to us, no mention is made of any changes in the shape of embryos and fetuses smaller than 30 cm.

Schultz (1919) did research on prenatal human material with a CBL from 5.4 to 28.6 cm. He found that after fixation, the sitting height decreased by an average of 2.54% (from +1.8 to −6.7%), the head length increased by an average of 0.88% (from −6.8 to +13.5%) and the head width increased by an average of 4.83% (from −4.4 to +13.5%). Thus, we could increase the CBL by 2.54%. However, because we are dealing with averages here, in this thesis the CBL will be reported exactly as measured.

Sizes of the Developing Orbit

In order to measure the increase in size of the parts of the orbital walls, we decided to measure the surface areas as described in the section on Methods and materials.

Apart from measuring surface areas, one might also measure linear distances and angles. In this thesis, the only linear distance reported is the distance between the center of the lacrimal fossa and that of the optic canal, because here there are no bony or soft structures in the orbital wall. Other linear measurements would not give a clear picture of the increase in orbital size because the parts of the developing orbital walls also shift in relation to one another in more than merely the linear direction. An example is the horizontal width of the orbital aperture between, on the medial side, the frontal process of the maxilla and, on the lateral side, the zygomatic bone. Both structures expand in the cranial direction, the lateral anterior margin being formed much later than the medial anterior margin. With regard to the vertical width of the orbital aperture between the cranial anterior margin and the caudal anterior margin, the cranial one is formed earlier than the caudal one. During development, the zygomaticomaxillary suture moves in

a lateral direction and the whole inferior margin moves in a rostral direction. Thus, the orbital margins move in various directions in relation to each other and hence provide no comparable measure for the increase in orbital size. Only those structures that move away from each other in a straight line provide useful reference points for determining the proportional increase in the developing eye socket in the linear direction. Of these reference points, we know only the lacrimal fossa and the optic canal, the location of the lacrimal sac and the optic nerve, respectively, in the orbital wall.

The soft structures in the orbital cavity are also unsuitable for measuring the proportional increase in orbital size because they move in more than one direction in relation to the orbital wall. The eye, for example, not only becomes larger but also undergoes a marked change in position. The optic nerves are at an angle of 180° in embryonic stage 16, 84° in stage 23, and 55° from a fetus of CBL 65 mm until term. The lines through the anatomical eye sockets, from the center of the pupil to the center of the optic nerve disc, form an angle of 180° in stage 16 and one of 30° in adults.

We also measured the angle between that part of the frontal bone that constitutes the roof of the orbit and the part of the frontal bone that constitutes the lateral orbital wall, this being the craniolateral boundary of the 'regio craniolateralis.' The change in this angle is a reflection of the change in shape of part of the orbit and not of an increase in size.

Occasionally, the width of a primordial suture has been mentioned. However, the development of sutures is no indication of an increase in orbital size.

Only the non-osseous or soft structures in the orbital wall itself and particularly the tear ducts in the lacrimal fossa (see stages 16 and 22) and the dorsal part of the eye stem, and later the optic nerve in the optic canal, are reliable reference points between which the linear increase in size of the orbital walls can be measured. The medial wall between the center of the lacrimal fossa and that of the optic canal remains practically vertical during development. [The author refers here to his Fig. 1, a transverse section through the right orbit of a 75 mm CBL fetus at the level of the optic nerve, enlarged 11 times; the medial wall of the primordial orbit is bent slightly in the nasal direction. The distance between the center of the primordial lacrimal fossa and the center of the primordial optic canal, as reliable reference points, is reported as the only linear measurement.]

Artefacts during Slice-Making

The method of preparing slices is described in the Methods section. In brief, this comes down to cutting the slices without pause and continually rinsing the nitrocellulose block with 70% alcohol to prevent shrinkage of the block. The nitrocellulose slices were numbered and placed on top of one another in 70% alcohol in order to prevent dehydration and the subsequent shrinkage of the slices. [The author refers here to his Fig. 2, a frontal section through the head of a 76 mm CBL fetus, enlarged 3.76 times; the upper and lateral edges of the nitrocellulose block are straight and the angles at the top are 90°; there are no signs of shrinkage; only the lower edge is a bit irregular.]

Artefacts during Photographic Magnification

Spherical aberration during photographic magnification was prevented by using diaphragms so that only the central part of the lens was used. The photographic magnification was determined by photographing the slice and an object micrometer in an identical manner on the same film. In the figure the calibration shows no aberration. [The author refers here to his Fig. 3, a frontal section through the orbit of a 113 mm CBL fetus, enlarged 10.5 times; the calibration revealed no aberration.]

CONCLUSIONS

The orbit is a cavity and we have chosen the boundaries of this cavity as the subject of our study. We know that the bony parts that limit the orbit are also part of a much larger section of the skull. In describing the development of the orbit, we have restricted ourselves as much as possible to the study of the orbital wall, which consists partly of osseous structures.

This research on the prenatal development of the human eye socket has clearly shown that development is more than simply growing larger (the idea of the homunculus has been abandoned long since!). After all, significant changes take place during development: cartilage, bone and muscular structures originate from connective tissue and change not only in composition but also in size, shape and location, partly because of mutual differences in growth rate of the cartilage, bone and muscular structures involved in the orbital wall.

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In the early embryonic stage 16 (8–11 mm CBL, 37–42 days after ovulation), the primordium of the eye is present; however, neither cartilage, bone or muscular tissue of the eye socket can be identified. Around the primordium of the eye there are only vaguely defined condensations of the mesenchyme, in which the cartilage, bone and muscular tissue will later develop. There is no orbit yet, and therefore, in this period, we refer to the '*regio orbitalis.*'

Also in stage 22 (23–28 mm CBL, 54–56 days after ovulation) and even in the stage of 42 mm CBL (65–75 days after ovulation) one cannot yet speak of an orbit because the cartilage, bone and muscular structures are still separated from each other by undirected connective tissue. Moreover, this connective tissue cannot yet be distinguished from the orbital contents.

The term '*primordial orbit*' cannot be used until fetal stage 76 mm CBL (95–105 days after ovulation), when the complex of cartilage, bone and muscular tissue is clearly distinguished from the tissue of the orbital contents by differences in structure. The transition from the 'regio orbitalis' to the primordial orbit thus takes place between fetal stages 42 and 76 mm CBL. Even the orbit of a neonate is not yet fully grown and should still be called primordial because (1) almost 50% of the floor is formed by Müller's muscle, (2) there is still non-ossified connective tissue, especially in the apex, and (3) the ethmoid bone is not yet completely ossified.

Models have been made in order to visualize the developmental stages of the orbit in an understandable way and in order to obtain insight into the metamorphosis of the eye socket. Explanatory drawings provide an overview of the increase in area of the orbital surfaces. Measurements of the models and the explanatory drawings, as well as microscopic studies of the slices, have shown that: (1) initially, the developing orbit is vaguely indicated by condensations in the mesenchymal connective tissue area; (2) in this connective tissue area, chondral, osseous and muscular structures develop and grow until, in the fully developed stage, the orbital content is surrounded by bony surfaces with a thin layer of connective tissue as periosteum, and by a muscle fragment; (3) the embryonic and early fetal phase, during which one can only speak of a 'regio orbitalis,' is followed by a period in which we can speak of a primordial orbit; (4) the phase of the primordial orbit extends until after birth; (5) the surface area of all orbital walls increases more or less linearly; (6) the 'musculus orbitalis Mülleri' occupies a special place in the orbital wall; (7) the so-called 'regio craniolateralis' is the primordium, which, in the fully developed stage, is occupied by the thick intersection of the frontolateral and the horizontal part of the frontal bone; (8) in frontal slices, the shape of the primordial orbit, as well as that of the fully developed orbit, is more or less round; (9) the prenatal development of an eye socket is a complex event, characterized by changes in composition, shape and size of the orbital wall; and (10) the orbit can only be denoted by the term "eye socket" when it is fully developed.

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