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Growth Curves, Measurement and Three-Dimensional Reconstruction in the Histopathology of Temporal Bones

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Key Words

Temporal bone · Stapes · Saccule · Utricle · Cochlea · Regression analysis · Growth curve · Mitscherlich model · Three-dimensional computer reconstruction · VATER syndrome

Abstract

The paper consists of three parts, in which the opportunities for measurement in histopathology of the temporal bone are shown. Measurement of size and distance (1D measurement) is used for determining the growth curves during the fetal period. Measurement of area (2D measurement) shows a difference in volume of mesenchymal tissue in the middle ear cavity between the groups with and without kidney pathology during the prenatal period. A computer 3D reconstruction of malformations of the middle and inner ears can be applied in understanding the pathology of the hearing organ. The first part concerns the application of measurements of size and distance to a set of 121 temporal bones from 71 fetuses. For evaluation, the following parameters were collected from the histological samples: the height and width of the stapes, the height of the vestibule, the length of the macula sacculi, the length of the macula utriculi, and the height and width of the cochlea. Regression analysis was applied to the data set to determine the relationship of the growth curves of particular structures. The data for growth curves from 36 aborted, presumably normal fetuses, are used. So the growth curves show the normal development of these structures. The Mitscherlich model was found to be the most satisfying for the growth curve in the fetal period. The amount of mesenchymal tissue in the middle ear (2D measurement) was determined after computer digitalization (Lucia) of the samples' area of the middle ear cavity. Statistical tests confirmed a larger amount of this tissue in the group of samples obtained from fetuses with pathological kidney findings (agenesis of kidneys, Potter's sequence), in comparison with a group of spontaneously aborted fetuses without kidney damage.The 3D computer reconstruction of the temporal bone, the third topic, was created using our own Medicus software written for the project. In a sample of bone without pathology, reconstruction enabled assessment of basic anatomical structure and two temporal bones show malformation of the stapes (VATE syndrome) and the cochlea (trisomy 21).

Wachstumskurven, Vermessungen und 3-D-Rekonstruktionen von histologischen Schnitten des Felsenbeins

Dieser in drei Teile gegliederte Artikel zeigt, welche Art von Messun gen an histopathologischen Schnitten des Felsenbeins durchgefüh werden können. Distanzmessungen (eindimensionale Messungen ermöglichen es, Wachstumskurven während der Fetalperiode z erstellen. Ausmessungen von Flächen (zweidimensionale Messun gen) dienen dazu, Unterschiede des mesenchymalen Gewebes in Mittelohr während der pränatalen Entwicklungsperiode aufzuzei gen – dies bei Verlgeichsgruppen mit und ohne Nierenpathologie Eine am Computer erzeugte 3-D-Rekonstruktion von Missbildunge an Mittel- und Innenohr dient dem Verständnis der Pathologien de Hörorganes. In einem ersten Teil wurden an 121 Felsenbeinen vo 71 Feten Grössen- und Distanzmessungen durchgeführt. Folgend Parameter wurden an den histologischen Schnitten evaluiert: Höh und Breite des Stapes, Höhe des Vestibulums, Länge der Macul sacculi, Länge der Macula utriculi, sowie die Höhe und Breite de Cochlea. Die ermittelten Daten wurden einer Regressionsanalys unterzogen, um die Wachstumskurven verschiedener Gewebestruk turen zu untersuchen. Die Standard Wachstumskurve errechnet sich anhand 36 abortierter Feten mit mutmasslich normaler En wicklung. Das Mitscherlich-Modell erwies sich als das hilfreichst Rechenmodell für Wachstumskurven in der Fetalperiode. Die com puterunterstützten Volumenmessungen des mesenchymalen Ge webes im Mittelohr zeigten eine statistisch signifikant vermehrt Gewebemenge bei Feten, die unter einer Nierenerkrankungen (z.E Nierenagenesie oder Potter-Syndrom) litten, im Vergleich zu eine Gruppe Feten ohne Nierenerkrankungen. Die 3-D-Messungen wu

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den mit Hilfe der speziell für dieses Projekt entwickelten Software «Medicus» durchgeführt. Die 3-D-Rekonstruktion eines Felsenbeines ohne Anomalien diente als Referenz, um missgebildete Felsenbeine zweier Feten zu studieren. Beim einen lag eine Missbildung der Stapes (Vater-Syndrom), beim andern eine Missbildung der Cochleae (Trisomie 21) vor.

Reconstruction tri-dimensionnelle de coupes histologiques d'os temporaux pour diverses mesures et l'évaluation de la croissance

Cet article en trois parties montre les mesures qu'il est possible de faire sur les coupes histo-pathologiques de l'os temporal. Des mesures de distance, en une dimension, permettent d'établir des courbes de croissance de diverses structures durant la période fœtale. Des mesures de surface, en deux dimensions, permettent de mettre en évidence des différences dans le volume de tissus mésenchymateux de l'oreille moyenne entre des groupes de sujets avec ou sans atteinte rénale durant la période de développement pré-natal. Des reconstructions tri-dimensionnelles computérisées de malformations de l'oreille moyenne et de l'oreille interne peuvent être utiles à la compréhension des pathologies des organes de l'audition. Des mesures de distance ont été réalisées sur 121 os temporaux provenant de 71 fœtus. Ont été mesurées la hauteur et la largeur de l'étrier, la profondeur du vestibule, la longueur des macules du saccule et de l'utricule, la longueur et la largeur de la cochlée. Une analyse de régression a été utilisée pour déterminer la relation des courbes de croissance de diverses structures. La croissance a été évaluée sur la base de 36 fœtus avortés, présumés normaux. Les mesures du volume de tissus mésenchymenteux dans l'oreille moyenne faites par analyse computérisée d'échantillons de tissus montrent que la quantité mésenchymenteux est statistiquement plus élevée chez les fœtus souffrant d'une pathologie rénale telle qu'une agénésie, par exemple, en comparaison avec une groupe de fœtus avortés, sans atteinte rénale. Les mesures tri-dimensionnelles ont été réalisées à l'aide d'un logiciel Medicus, spécialement écrit pour le projet. La reconstruction tri-dimensionnelle d'un os temporal sans anomalie sert de référence pour l'étude des malformations de deux os temporaux de fœtus souffrant de malformations, l'une de l'étrier dans le cadre d'un syndrome de Vater, et l'autre de la cochlée, dans une trisomie 21.

Introduction

Temporal bone histological findings can be evaluated from several points of view. Primarily, particular pathological findings are systematically classified into appropriate groups according to development or genetic damage. For a particular syndrome, the appropriate set of histological findings should be evaluated by statistical methods to create and to test valid hypotheses. Knowledge of the histopathology of those who have died and who suffered from both damage to the auditory system and developmental or genetic defects can be applied in the treatment of living patients.

Secondarily, besides this traditional methodological approach, statistical analysis and computer reconstruc-

Table 1. Titles of measures

1	Height of stapes
2	Width of stapes (footplate)
3	Minimal distance between footplate and saccule
4	Macula of saccule
5	Macula of utricle
6	Height of cochlea
7	Width of cochlea

tion may be applied to obtain deeper knowledge. example, the determination of the size of the middl ossicles enables designing prostheses for use in middl surgery. Knowledge of the relationship and distances ween stapes and other structures of the inner ear (ut saccule) influences surgery of the stapes [1].

Other important information may be obtained f the measurement of the area of mesenchymal tissue i middle ear and of the whole middle ear cavity, provi data on the embryological development and patholo changes of the middle ear [2, 3].

Recently, there has been an increasing interest in th dimensional (3D) reconstruction in the otologic f Human temporal bone histological sections are the suitable materials for this reconstruction. 3D comp reconstruction provides an easier view of the mutual tial relationships between structures of the middle an inner ear and the facial nerve. Besides the assessmer anatomical relationships, it is also possible to use a reconstruction to show abnormalities of the middle inner ear. In 1993 Harada et al. [4] published the paper on 3D reconstruction of the anomalous temp bone from a case of trisomy E syndrome. Knowledg malformation of middle ear ossicles will affect the typ tympanoplasty during middle ear surgery and the pat ogy of the shape of the cochlea will affect tactics of chlear implantation.

Methods and Results

The temporal bones were removed within 24 h after abo After fixation in 10% formaldehyde solution, specimens were d cified in 10% formic acid, embedded in paraffin wax and secti strictly horizontally (midmodiolar plane) at a thickness of 10 Every tenth section was stained with hematoxylin-eosin and e ined microscopically.

Growth Curves (1D Measurement)

The measures shown in table 1 (fig. 1–3) were obtained in a 121 temporal bones from 71 fetuses from the 13th to 42nd we gestation. A microscopic ruler with a precision of 0.01 mm was The measures were used as the criterion of growth of the obse structures of the middle and inner ears with relation to the a fetuses. The age of fetuses was determined on the basis of know of the week of gestation and weight and height of the fetuses.

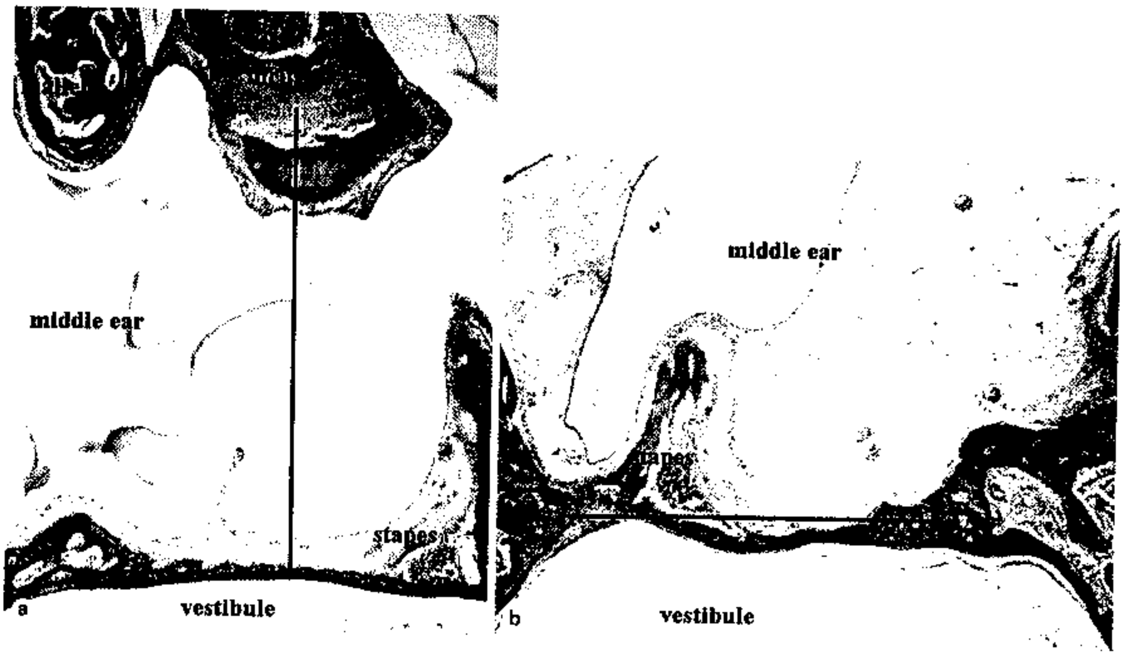

Fig. 1. Histological picture of the height (**a**) and width (**b**) of stapes.

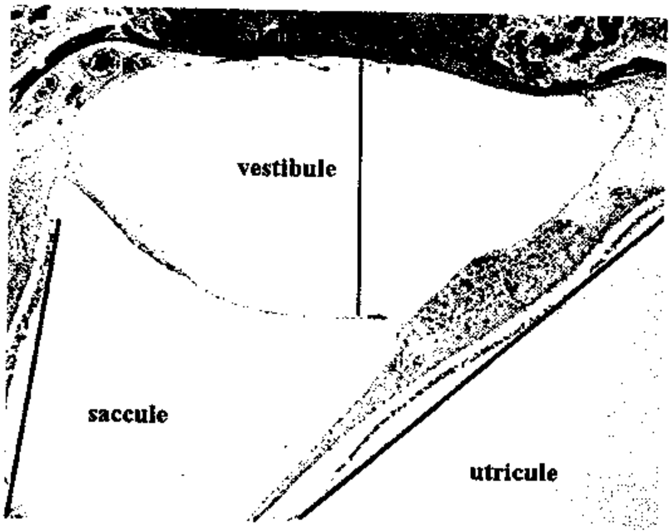

Fig. 2. Histological picture of the distance between footplate and saccule, macula of saccule and macula of utricle.

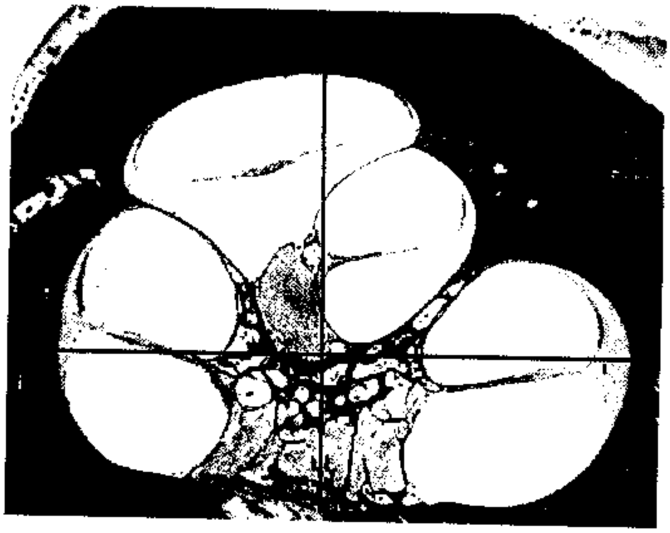

Fig. 3. Histological picture of cochlear height and width.

authors are aware of possible inaccuracies in the measurement of particular measures due to the processing of every tenth section, which might affect the results. Another factor that influenced accuracy was the difficulty of ensuring the ideal horizontal plane of cutting of the temporal bone. Therefore only the data of temporal bones that were sectioned in strictly horizontal plane were used.

The set of 127 temporal bones with measures obtained from 71 fetuses was divided into two groups. The first group included fetuses with physiological development (36 fetuses), while the second one included fetuses with retardation of development (35 fetuses) on the basis of data obtained from the Departments of Genetics and of Pathology. Retardation of development was determined on the basis of the information on the fetus and newborns: its age, height, weight and gestational age. Measures only from fetuses with physiological development were used in regression analysis, and the results are shown in graphs of the regression growth curves (fig. 4–10). A computer-assisted nonlinear regression analysis of diagnostics enabling simultaneous examination of data was carried out. A growth curve model was proposed, and a mathematical method with Ratkowski criteria was applied to find the best descriptive model of the height of 40 stapes from 27 fetuses versus time. The regression search among 13 growth models was discussed in our previous paper [5]. The best fit was achieved with the Mitscherlich model [5] for normal development of middle and inner ear structures (fig. 4, height of stapes; fig. 5, width of stapes; fig. 6, footplate-saccule; fig. 7, macula sacculi; fig. 8, macula utriculi; fig. 9, height of cochlea; fig. 10, width of cochlea).

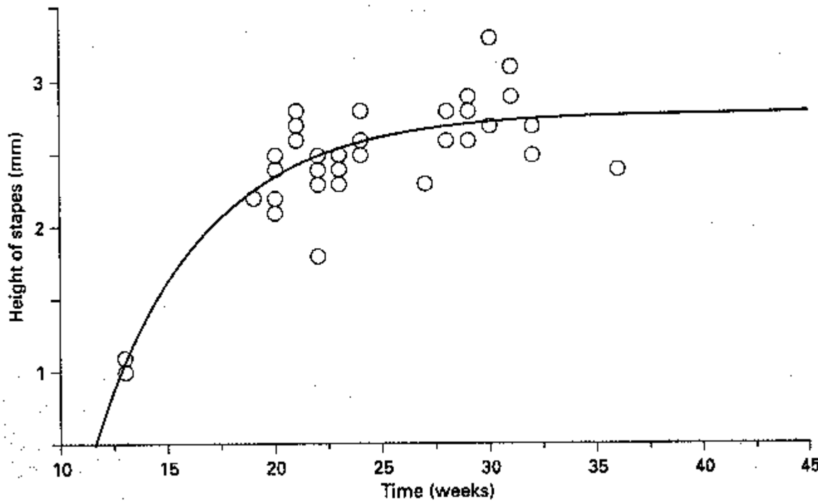

Fig. 4. Mitscherlich model of the growth curve concerning the height of stapes (mm) dependent on gestation time (weeks).

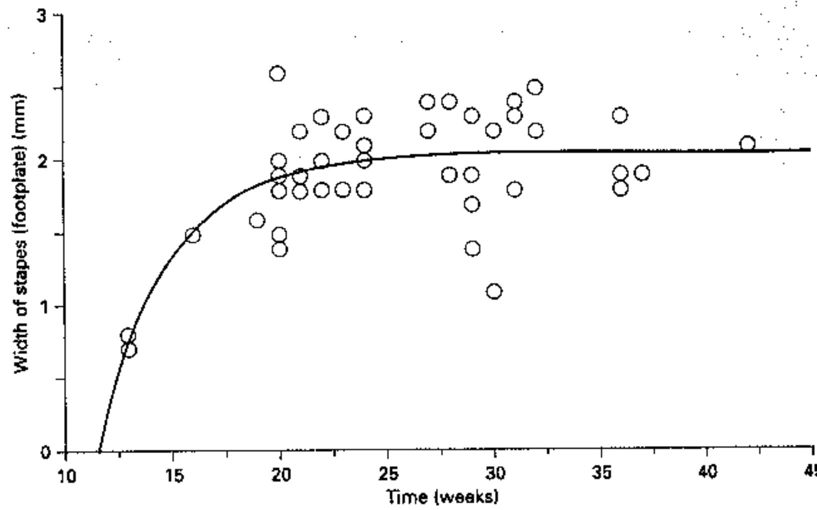

Fig. 5. Mitscherlich model of the growth curve concerning the width of stapes (mm) dependent on gestation time (weeks).

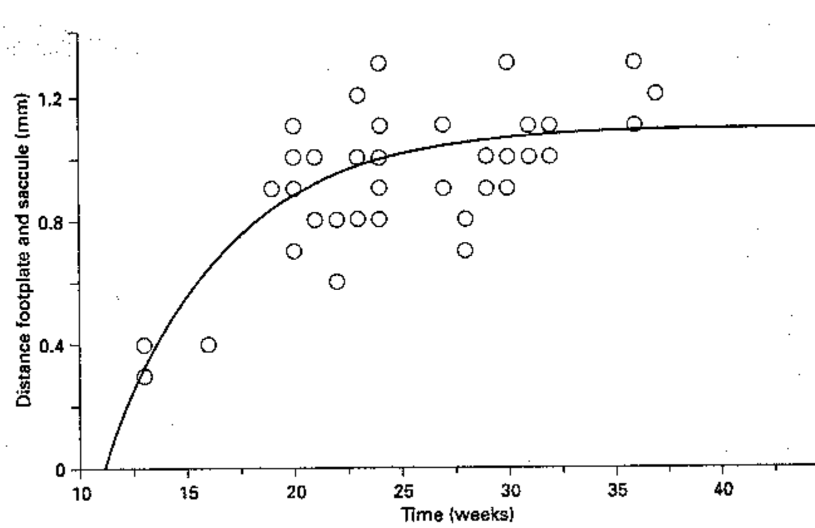

Fig. 6. Mitscherlich model of the growth curve concerning the distance footplate of stapes and saccule (mm) dependent on gestation time (weeks).

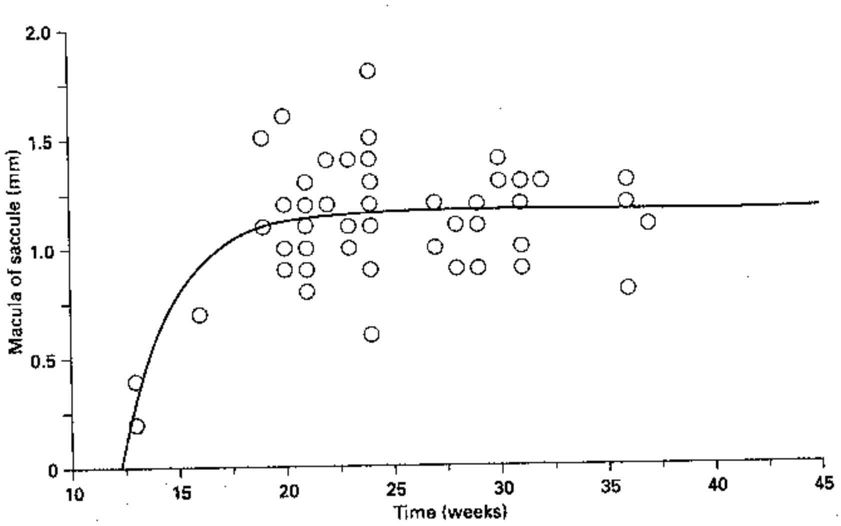

Fig. 7. Mitscherlich model of the growth curve concerning the macula of saccule (mm) dependent on gestation time (weeks).

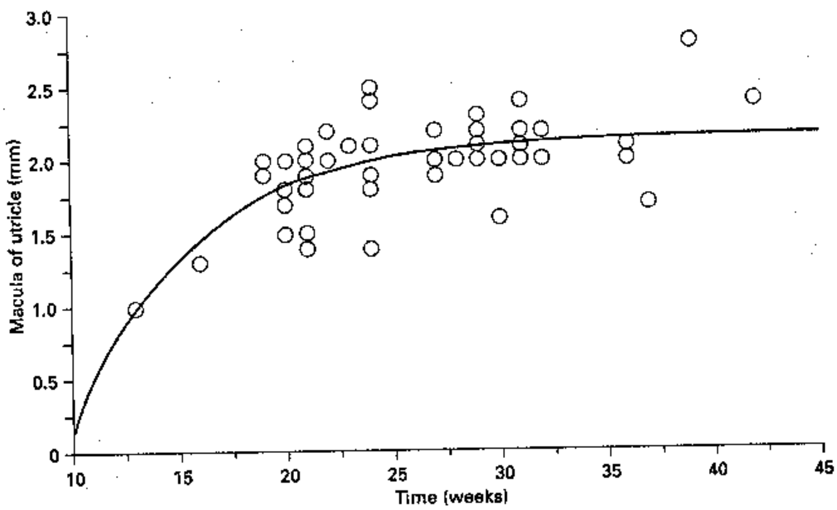

Fig. 8. Mitscherlich model of the growth curve concerning the macula of utricle (mm) dependent on gestation time (weeks).

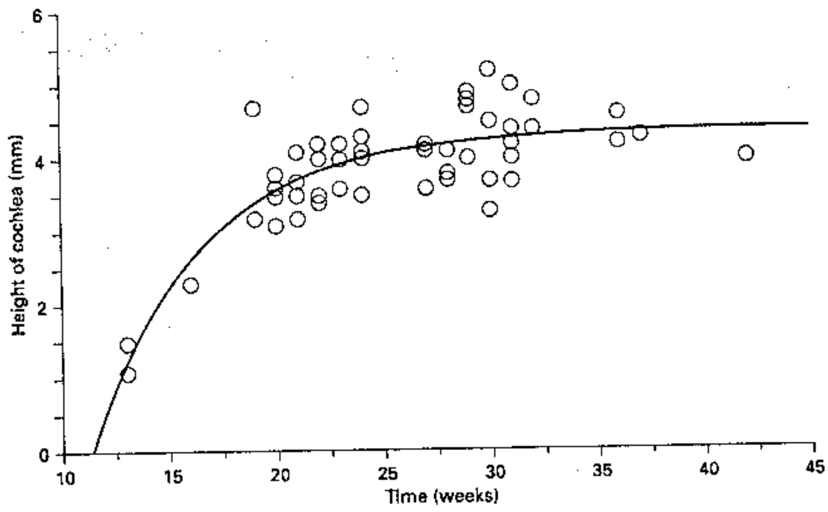

Fig. 9. Mitscherlich model of the growth curve concerning cochlear height (mm) dependent on gestation time (weeks).

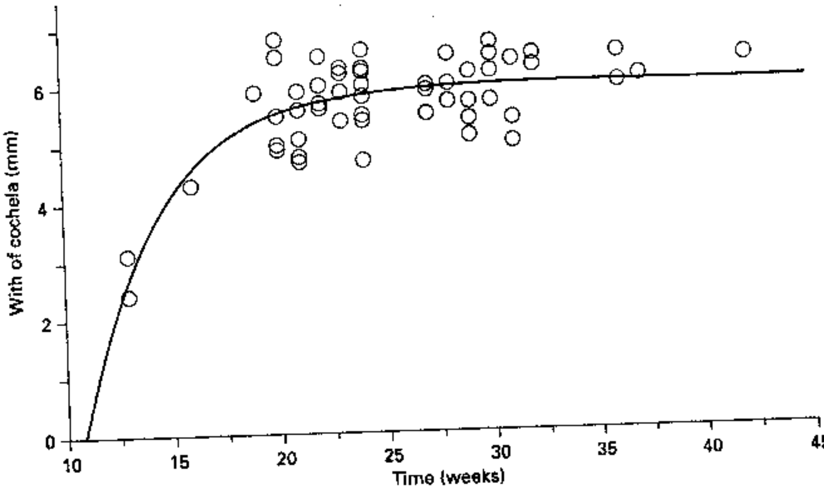

Fig. 10. Mitscherlich model of the growth curve concerning cochlear width (mm) dependent on gestation time (weeks).

Table 2. Percentage of mesenchymal tissue in the middle ear cavity dependent on the weight of fetuses

Group	Fetal weight			
	0–750 g	751–1,000 g	1,001–1,500 g	1,501 g and more
Renal agenesis	88.8	85.3	–	79.8
Severe renal pathology	80.1	78.8	–	–
Mild renal pathology	81.4	13.4	–	–
Controls	81.1	66.1	38.4	0.8

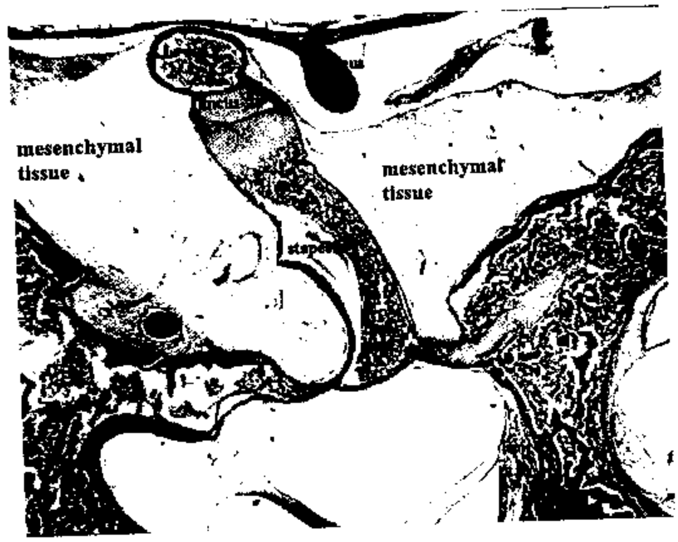

Fig. 11. Histological picture of the middle ear cavity with mesenchymal tissue.

The maximum rate of growth of the height and width of stapes was observed in the 13th to 24th weeks of pregnancy. The average height and width of stapes are 1.05 and 0.75 mm, respectively, in the 13th week, and 2.62 and 2.01 mm in the 24th week. The later growth of stapes after the 25th week is slower, and the average height and width in the 30th week are 3.02 and 2.07 mm, respectively.

Minimal changes in distance between the footplate and stapes were found after the 25th week of gestation, in the length of the macula sacculi after the 20th week of gestation and in the length of the macula utriculi after the 28th week of gestation. The average height of the cochlea before birth was 4.23 mm, and the width of the cochlea 5.84 mm.

Measurement of the Area of Mesenchymal Tissue in the Middle Ear Cavity

Measurement of an area is an example of two-dimensional assessment. The authors focused on the determination of the area of the middle ear, which is filled with mesenchymal tissue during fetal growth. For the embryonal development of the middle ear cavity, it is characteristic that the mesenchymal tissue disappears following pneumatization of the middle ear cavity. In the group with kidney damage a decrease in mesenchymal tissue was noted. The results were compared with a group of spontaneously aborted fetuses without kidney damage.

Thirty-seven temporal bones obtained from 22 fetuses aged from 19 to 37 weeks of gestation were analyzed [6]. In 5 fetuses agenesis of kidneys was diagnosed, in 6 Potter's sequence (a serious functional kidney damage), and in 2 fetuses other, less serious functional pathology (kidney dystopia) was found. A comparison was made with a set of 9 fetuses spontaneously aborted without pathology in the kidney or urinary system.

Each temporal bone was measured in all cuts where the stapes was located (fig. 11). This part of the middle ear cavity was chosen for two reasons: (1) it is a well-defined area of the middle ear cavity; (2) in the lateromedial direction it has the largest volume. On the histological specimen, both the whole middle ear cavity and the part with mesenchymal tissue were measured using the program Lucia-M. Table 2 shows the percentage of mesenchymal tissue in the middle ear cavity. A relative expression of mesenchymal tissue was more appropriate because absolute values could be biased by the fact that the plane of the cut was not ideal within the whole set. The percentage of mesenchymal tissue in the middle ear cavity was used, so it was possible and easier to measure the computed areas, and the use of the stereological technique to arrive at volumetric figures was not necessary.

A larger amount of mesenchymal tissue was found in the middle ear cavity in the group with agenesis of kidneys or Potter's sequence in comparison with the groups with kidney dystopia (a less serious functional pathology) or those with no pathology of the kidney or urinary system. The group with a less serious functional kidney pathology showed a severe drop of mesenchymal tissue just as the group without kidney pathology. In the group with no pathology of the renal/urinary tract, the volume of mesenchyme decreased during fetal development. Mesenchyme occupied 70–80% of the middle ear cavity in the 19th–20th weeks gestation but only 1% in the 37th week. This decrease appears to be a normal process of development.

The nonparametric method of statistical analysis was used. At the usual significance level $\alpha = 0.05$ the hypothesis of the measures of both groups being equal to the theoretical numbers may be rejected. It suggests that there is really a different volume of mesenchymal tissue in the middle ear cavity between the groups with and without serious kidney pathology.

3D Reconstruction

The 3D reconstruction consists of several steps. In the first step the particular histological cuts are digitized. In the second step the digitized cuts are put into a common orthogonal space. The data are transformed in such a way that the reference points correspond in all cuts. This transformation usually requires rotation and shift, a process called registration. For this part of the project our own software, Pix, enabling manual registration of cuts was written [7, 8]. The registered images of histological cuts were composed to the model in the volume representation based on voxels.

In the next step, called segmentation, selected structures in the volume model are marked using the Analyze system, version 6.2. [9]. For these purposes two means of segmentation were employed – the manual draw of the edge of the structure and the automatic threshold based on the different intensity of gray color dependent on the properties of the structures. During segmentation different colors were assigned to the structures of interest for their better resolution.

The final step, rendering, visualizes the volume model constructed in previous steps. Rendering was performed using our own Medicus software, which visualizes data in both planar (2D) and spatial (3D) modes using different methods of rendering [7]. Selected structures in the volume model can be viewed from different positions. The user can set the parameters of displayed structures such as mode of view, orientation, size, color, opacity or partial cuts.

The 3D reconstruction methodology was used for rendering the inner ear and middle ear ossicles and the facial nerve of the right temporal bone without any pathology (fig. 12, 13) and the right temporal bone with malformation of stapes (fig. 14) in a case with VATER syndrome (C. Northrop, Temporal bone foundation, Boston). The anterior crus had a normal connection with the footplate, but the posterior part of the footplate was missing and the posterior crus had no connection with the footplate and the bone of the middle ear. The posterior crus was found in the sinus tympani. In our previous study [10] there was a 3D reconstruction of the cochlea of a fetus with trisomy 21 syndrome and Mondini dysplasia, which is manifested by only 1.5 coil of the cochlea.

Discussion

Growth Curves (1D Measurement)

The mathematical model was found for growth curves of temporal bone structures. The best fit was achieved with the Mitscherlich model. From the assessment of the growth curves it can be concluded that most of the development of the observed parameters is completed before birth. But when the height and the width of stapes from the fetal period are compared with those of adult stapes [11], it is possible to find growth of stapes after birth. Schuknecht [11] published the average height and width of adult stapes as 3.26 and 2.99 mm. The adult stapes showed considerable variation in size, the minimum sizes being 2.56 and 2.64 mm and the maximum sizes 3.78 and 3.36 mm, respectively. Small differences after birth may be explained by variable plane of section between specimens; the growth curves support the literature, which describes the temporal bone and its contained structures as fully developed at birth.

In their work, Anson and Harper [12] obtained for the distance between the footplate of stapes and saccule an average value of 1.4 mm and a minimal value 0.8 mm. According to Pauw et al. [1], the distance from the center of the footplate to the saccule ranges from 1.7 to 2.1 mm. The average distance between footplate and saccule was 1.02 in our cases before birth. Knowledge of this distance is important for stapes surgery of adult cases.

Anson and Harper [12] reported the maximum length of the macula utriculi to be 2.3 mm and its width 2.1 mm; the size of the macula of saccule was 2.2 mm in length and 1.2 mm in width. It has to be stressed that the sizes of the macula of saccule (1.15 mm before birth) and utricle (2.05 mm before birth) that were obtained in this paper represent the maximum length in the horizontal plane of the temporal bone. With respect to the spacial localization of the macula it is not possible to take these measurements as the sizes of the structures mentioned above. However, it is possible to use these results for growth curves.

Anson and Donaldson [13] noted that the growth of the cochlea is completed by the 21st week. The growth curves of the height and width of the cochlea in our cases show a rapid development of the cochlea before the 20th week of pregnancy. While growth of the cochlea was minimal after the 21st week of gestation, the increase in height seems to be bigger than the increase in width during this fetal period.

Measurement of the Area of Mesenchymal Tissue in the Middle Ear Cavity

Pneumatization of the middle ear cavity starts from the eustachian tube and continues along the tympanic membrane to the lateral part of the mesotympanum. The

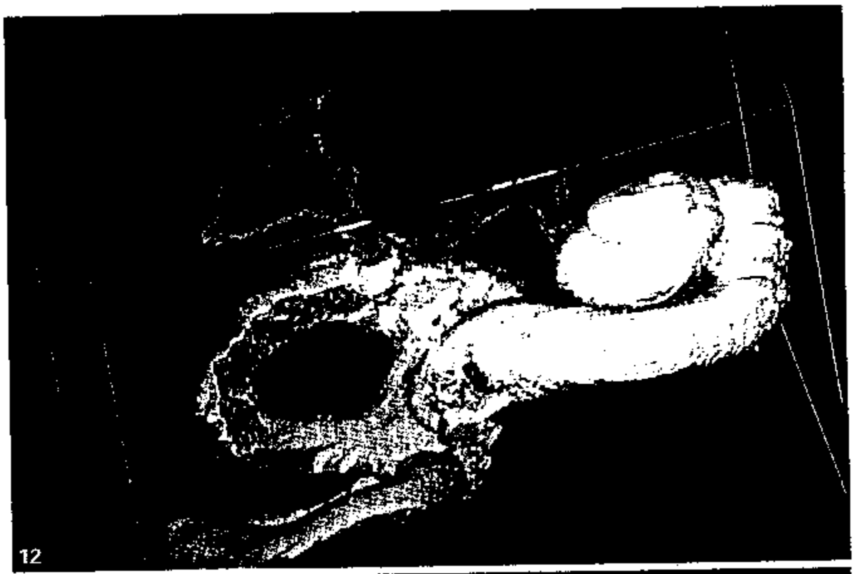

Fig. 12. 3D reconstruction of bone labyrinth (yellow, blue), ossicles (malleus dark blue, incus violet, stapes yellow) and facial nerve (red), view on the lateral and inferior side.

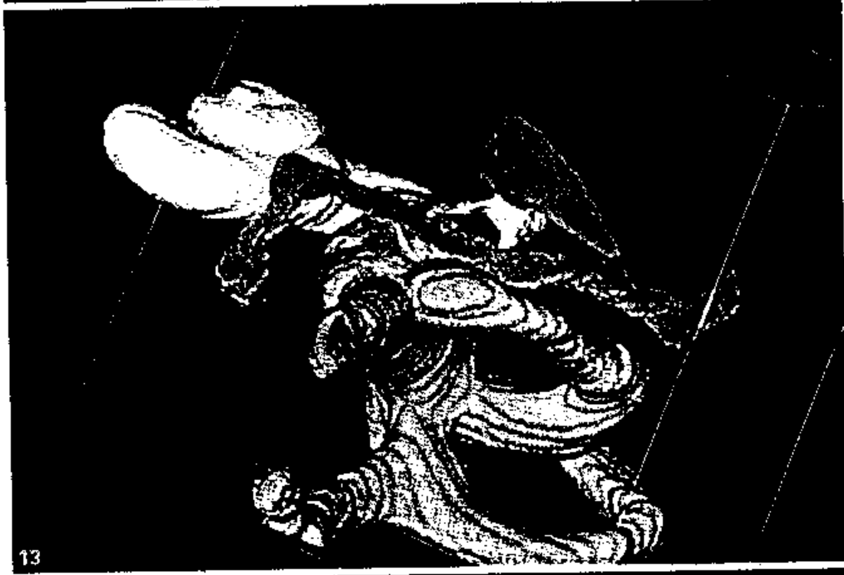

Fig. 13. 3D reconstruction of bone labyrinth (yellow, blue), ossicles (malleus dark blue, incus violet, stapes yellow) and facial nerve (red), view on the superior side.

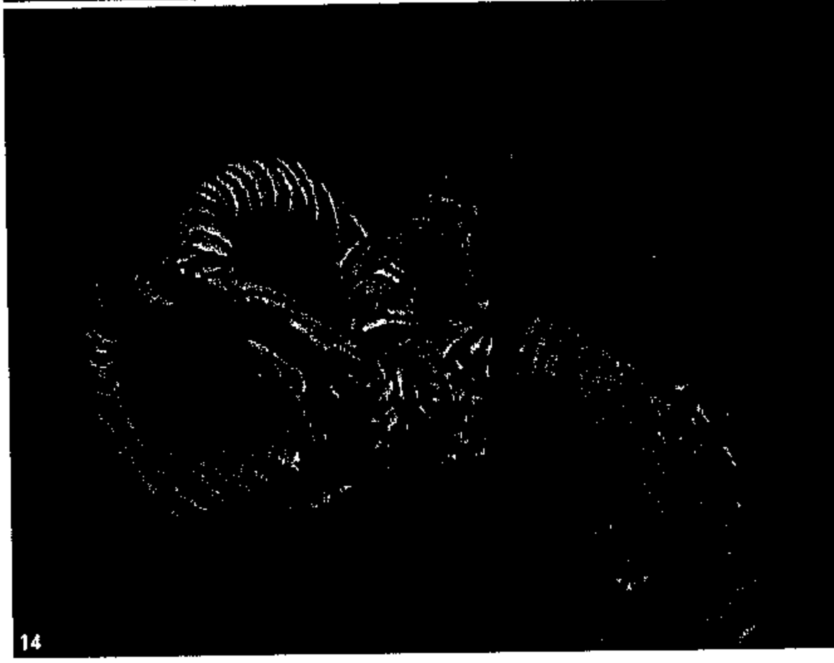

Fig. 14. 3D reconstruction of cochlea and ossicles with malformation of stapes (yellow) in VATER syndrome.

mesenchyme remains longer in the medial part of the mesotympanum around the stapes. The decrease of mesenchymal tissue in the middle ear cavity may be explained by different theories – absorption, resorption or halted growth of mesenchymal tissue with parallel growth of the middle ear cavity [3]. Mesenchyme normally occupies less than 20% of the middle ear cavity at birth and most of it disappears by 1 year of age. In the middle ears of individuals with congenital anomalies, mesenchyme occupies about 30% or more of the middle ear cavity at birth and does not disappear until about 3 years of age. The prevalence of a larger amount of mesenchymal tissue in the group of fetuses with kidney damage, most frequently with Potter's sequence or other malformations, has been reported by many authors [14–16]. Theoretically, it is explained by the absence of either amniotic fluid or another factor produced by kidney, which have influence on the growth and maturation of lung tissue and also of the middle ear cavity as an embryological part of the respiratory tract [17].

Statistical analysis of this set confirmed the prevalence of a larger amount of mesenchymal tissue in the group of fetuses with serious kidney damage in comparison with spontaneously aborted fetuses and fetuses with less serious functional kidney pathology. The fetus with renal pathology had no typical malformations of the hearing organ.

3D Reconstruction

A 3D reconstruction of the anatomically normal bone was prepared for exploration of the relationships of the chosen structures [4, 18].

The present 3D reconstruction of the temporal bone was performed on normal temporal bone specimens for the purpose of obtaining more accurate knowledge of normal anatomy. In the case of malformation of the hearing organ, the diagnosis is easier due to the possibility of observing structures as objects in space. 3D reconstruction in 1 case with malformation of stapes and in 1 case with Mondini dysplasia of the cochlea was carried out. VATER syndrome was described by Quan and Smith [19] as an association of anomalies with the acronym VATER including vertebral, anal, tracheal, esophageal and radial limb anomalies in 1973. Sakai et al. [20] were the first to study the temporal bone in VATER syndrome and found anomalies including reduced size of the motor and sensory components of the facial nerve, large endolymphatic duct and sac, and minor abnormalities in the configuration of the stapes and vestibular system. Malformation of the stapes, partial absence of the posterior crus and posterior part of the footplate were demonstrated in 3D reconstruction in this paper.

Conclusion

The use of computer-assisted mathematical methods brings opportunities for assessment of temporal bone histological findings. The measurement of the size and distance of structures of the hearing organ and the temporal bone helps design surgical tools, and both middle ear and inner ear implants. Measurement of the surface of the middle ear cavity on histological specimens makes knowledge of the physiological and pathological development of the middle ear more accurate. 3D reconstruction augments knowledge of the structures of the ear, and can be used in surgery of the temporal bone. In particular, it is useful in cases of preoperative specimens with data obtained from CT for treating abnormalities of structures of the external, middle and inner ears.

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