The Time and Order of Appearance of Ossification Centers in the Hamster before Birth

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The time and order of appearance of the ossification centers of the skull, the axial skeleton, the appendicular skeleton, and the pectoral and pelvic girdles were studied in fetuses of the golden hamster. They were cleared with KOH and stained with alizarin red S by Dawson's method. The results obtained were as follows.

1. The first appearance of an ossification center in all the skeletons is in the clavicle at 11 days and 15 hours post coitus.
2. In the axial skeletons, the first ossification center appears in the cervical vertebral arches at 12 days and 15 hours. The vertebral bodies ossify at 13 days and 12 hours in the thoracic bodies somewhat later than the respective vertebral arches.
3. The anterior appendages begin to ossify a few hours earlier than the posterior ones.
4. The first ossification center in the skull appears in the mandible at 12 days. Before the time of birth all the skull bones except for the ethmoid begin to ossify.
5. The ossification of newborn hamsters seems immature when compared to those of the rat and the mouse in which more ossification centers are present at birth than in the hamster, especially in the manus and pes.—Exp. Animals, 23 (3), 113—122, 1974.

Introduction

The golden hamster, characterized by a shorter gestation period and more rapid development in the prenatal period than most of other laboratory mammalian animals, was studied as to osteogenesis by Beatty et al. [2] for the first time in 1950. In that study, they recorded the time of appearance of ossification centers and concluded that the appearance of ossification centers in the hamster was "explosive". Arsdell et al. [1] reported the ossification in the middle and internal ear of this animal. Friant [6, 7] histologically investigated the ossification of Meckel's cartilage. Recently, Rajtova [10] described the development of the chondrocranium in detail by the method of serial sectioning. As

for the development and the growth of bones in the postnatal life, a few studies were reported by Beyerlein et al. [3] and Schumacher et al. [12]. These studies give an outline of the development of bones in the hamster, but as to the time and order of appearance of ossification centers, the results were not conclusive.

In the present study, in order to get a standard of the bone growth in the hamster, the time and order of appearance of ossification centers in the fetuses were investigated in detail.

Materials and Methods

The fetuses of the golden hamster (*Mesocricetus auratus var cream*) used in this study were ob-

Table 1. Number of specimens used in this study.

Prenatal age days	hours	Number of litters	Number of specimens
11	14	1	11
11	15	1	12
11	21	1	12
12	00	2	24
12	04	1	9
12	10	1	13
12	12	1	10
12	15	1	11
12	18	3	32
12	21	1	12
13	00	2	20
13	03	1	12
13	12	1	10
13	15	3	27
13	20	1	10
13	23	1	9
14	00	3	44
14	05	1	11
14	12	1	9
14	15	1	14
14	18	1	11
14	23	1	12
15	00	2	26
15	06	1	11
15	12	1	14
at birth*		4	25
total		38	411

* The time of first birth of the four litters: 15 days 14 hours, one litter, 15 days 15 hours, two litters and 15 days 16 hours, one litters.

Table 2. The time of appearance of ossification centers in the vertebrae.

1.	Arches	earliest		mean,		latest	
		days	hours	days	hours	days	hours
	C.V. 1-7	12	12	12	15	12	21
	T.V. 1-2	12	12	12	15	12	21
	T.V. 3-5	12	15	12	21	13	12
	T.V. 6-9	12	18	13	00	13	12
	T.V. 10-13	12	15	12	21	13	12
	L.V. 1-4	12	15	12	21	13	12
	L.V. 5-6	12	18	13	00	13	12
	S.V. 1-2	13	00	13	15	13	20
	S.V. 3	14	00	14	05	14	12
	Ca.V.	14	23	15	06	at birth	
2.	Bodies						
	C.V. 1-5	15	00	15	06	at birth	
	C.V. 6-7	14	00	14	12	14	18
	T.V. 1-2	13	20	14	05	14	12
	T.V. 3	13	15	13	20	14	05
	T.V. 4-5	13	12	13	15	14	00
	T.V. 6-9	13	12	13	12	13	20
	T.V. 10-13	13	12	13	15	14	00
	L.V. 1-3	13	12	13	15	14	00
	L.V. 4-6	13	15	13	20	14	00
	S.V. 1-3	13	20	14	05	14	15

mean: the time when an ossification center was found in more than half individuals of a litter.

earliest: the time when an ossification center was found in at least one individual of a litter.

latest: the time when an ossification center was found in all individuals of a litter.

Explanation of abbreviations: C.V., cervical vertebrae; T.V., thoracic vertebrae; L.V., lumbar vertebrae; S.V., sacral vertebrae; Ca.V., caudal vertebrae.

tained from our closed colony descended from the colony of NBS*. The hamster exhibits regular estrus cycles with ovulation every four days and the onset of psychic estrus in females commences between 7 p.m. and 11 p.m. in our colony. Virgin females and the males were placed in mating cages at 8 p.m. every day and observed for copulation. The time of copulation was recorded after each mating which continued 30 minutes to 1 hour. Ovulation is considered to occur between 12 midnight and 1 a.m. [11]. The ovum is fertilized 6 to 12 hours after the ovulation, actual time of the fertilization being difficult to determine. Therefore, the days and hours of gestation were counted from the end of each mating. Four hundred and eleven fetuses from 11 days of gestation until birth (table 1) were cleared with KOH and stained with alizarin red S (alizarin sodium monosulphonate) by Dawson's method [5]. Twenty newborns from birth until 30 days of age were also studied for the purpose of reference.

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Observations

1. The Axial Skeleton (table 2, 3). The golden hamster has 42 vertebrae. The number of the cervical, thoracic, lumbar, sacral and caudal vertebrae are 7, 13, 6, 3, and 13 respectively.

As shown in fig. 1, ossification centers appear at first as two extremely small dots of staining in the cervical arches and the first two thoracic arches at 12 days and 15 hours. From the tenth arch to the fourth lumbar, they appear at 12 days and 21 hours, and from the sixth to the ninth thoracic arches and from the fifth to the sixth lumbar arches, they appear at 13 days. The first and second sacral arches ossify at 13 days and 15 hours, and the third sacral arch begins to ossify at 14 days and 15 hours. By 15 days and 6 hours the arches of the caudal vertebrae from the first to the third begin to ossify.

The ossification centers of the vertebral bodies, which have not appeared when the respective vertebral arches begin to ossify, are observed for the first time from the sixth to the ninth thoracic vertebral bodies at 13 days and 12 hours. The ossification of ver-

tebral bodies extends from that time forward to cervical region and backwards to the caudal.

The first five cervical vertebral bodies ossify at 15 days and 6 hours, the last among the

Fig. 1. Average ossification in the vertebral arches and bodies.

The lines indicate ossified area at birth and the dark portions inside the lines indicate the ossification at the prenatal age described in the figure. Explanation of the abbreviations: C.V., cervical vertebrae; T.V., thoracic vertebrae; L.V., lumbar vertebrae; S.V., sacral vertebrae; Ca.V., caudal vertebrae.

Table 3. The time of appearance of ossification centers in the sternum and the ribs.

		earliest		mean		latest	
		days	hours	days	hours	days	hours
Sternebra	1	14	05	14	12	14	15
	2-4	14	23	15	06	15	12
	5			after birth			
	6	14	15	14	23	15	06
Ribs	1	12	21	13	00	13	12
	2-13	12	12	12	21	13	03

Refer to Table 2. for explanations.

vertebral bodies.

The ossification centers of all the ribs except for the first one appear at 12 days and 21 hours. The first rib begins to ossify at 13 days. The first sternebra ossifies at 14 days and 12 hours. The sixth sternebra ossifies at 14 days and 23 hours, and the ossification centers of the second, third and fourth sternebrae at 15 days 6 hours. Only the fifth sternebra was recognized to be ossified after birth.

2. The Pectoral and Pelvic Girdles (table 4, fig. 2). The ossification center of the clavicle was observed at 11 days and 15 hours of gestation and this was the first ossification among all the bones. At birth, the ossified shaft of the clavicle is about 4mm long, whereas its epiphysis does not show any sign of ossification.

The ossification in the scapula occurs at 12 days and 10 hours. Two centers appear at first at the edges of the bone separately, which immediately fuse with each other to form a single wide plate. At birth, the plate is 3.5 mm in width.

The ossification of the ilium appears as a ring at 12 days and 15 hours in the diaphysis. It develops to 2mm long at birth. At 14 days and 5 hours, the ossification centers of the pubis and the ischium appear. At birth, the pubis is 0.8mm in length, and the ischium is twice the length of the pubis. The foramen obturatum is not formed at this time.

3. The Appendages (table 4, fig. 2). The

Table 4. The time of appearance of ossification centers in the bones of girdles and appendages.

	earliest		mean		latest	
	days:	hours	days:	hours	days:	hours
Clavicle	11	15	11	15	11	21
Scapula	12	10	12	10	12	12
Ilium	12	12	12	15	12	18
Ischium	13	23	14	05	14	05
Pubis	13	23	14	05	14	05
Anterior appendages						
Humerus	12	00	12	10	12	12
Ulna	12	10	12	12	12	12
Radius	12	10	12	12	12	12
Metacarpal						
2-4	13	23	14	05	14	12
5	15	12	at birth			
Distal phalanges						
1-5	13	20	13	23	14	05
Posterior appendages						
Femur	12	12	12	15	12	18
Tibia	12	12	12	15	12	18
Fibula	12	12	12	15	12	18
Metatarsus						
2-4	14	15	14	23	15	00
5	15	12	at birth			
Distal phalanges						
1-5	13	20	13	23	14	05

Refer to Table 2. for explanations.

Table 5. The time of appearance of ossification centers in the skull.

		earliest		mean		latest	
		days	hours	days	hours	days	hours
Mandible		11	21	12	00	12	10
Maxilla		12	10	12	12	12	15
Premaxilla		12	12	12	12	12	15
Exoccipital		12	12	12	15	12	21
Basisphenoid		12	12	12	15	12	21
Parietal		12	12	12	15	12	21
Frontal		12	12	12	15	12	21
Squamosal		12	12	12	15	12	21
Palatine		12	12	12	15	12	21
Basioccipital		12	18	13	00	13	12
Nasal		12	18	13	00	13	12
Vomer		12	21	13	00	13	12
Alisphenoid		13	00	13	03	13	12
Supra-occipital							
	13	00	13	03	13	12	
Tympanic		13	15	13	15	13	23
Interparietal		13	15	13	15	13	23
Zygomatic		13	15	13	15	13	23
Presphenoid		13	23	14	05	14	05
Basihyal		14	05	14	15	15	00
Lacrimal		14	05	14	15	15	00
Orbito-sphenoid							
	14	18	15	00	15	12	

Refer to Table 2. for explanations.

ossification of the humerus occurs at 12 days and 10 hours as a slender ring. At birth, the humerus is about 4mm long. It was observed that the ossification of the humerus precedes that of the ulna and the radius. At 12 days and 12 hours, the cylindrical ossification centers appear in the radius and the

ulna for the first time. The center of the radius is smaller than that of the ulna. At birth, the ulna is 3.8mm and the radius is about 3.0mm in length.

The ossification in the long bones of the hind limbs seem to start at the same time. At 12 days and 15 hours, the ossification cen-

Fig. 2. Average ossification in the girdles and the appendages.

Explanation of the abbreviations: fem, femur; fib, fibula; hum, humerus; ili, ilium; isch, ischium; m'car, metacarpus; m'tar, metatarsus; ph. dis, distal phalange; rad, radius; scap, scapula; tib, tibia; ul, ulna. Refer to Fig. 1 for further explanations.

ters of the femur, the tibia and the fibula were observed for the first time. The femur is developed to about 3.0mm in length at birth. The shaft of the fibula is more slender than that of the tibia. At birth, both of them are

2.8 mm in length.

In the distal five phalanges of the manus and the pes, the ossification centers appear at 13 days and 23 hours. At 14 days and 5 hours, the second, third and fourth metacarpi

Fig. 3. Average ossification in the skull.

Explanation of the abbreviations: a-sph, alisphenoid; tym, tympanic ring; b-hy, basihyal; b-oc, basisphenoid; b-sph, basisphenoid; exoc, exoccipital; zy, zygomatic; mx, maxilla; o-sph, orbitosphenoid; pal, palatine; sq, squamosal; vom, vomer.

begin to ossify, preceding the ossification of the metatarsus which begins a little later. By the time of birth, the fifth metacarpus and metatarsus begin to ossify, and no other ossification centers appear at all.

4. The Skull. Table 5 shows the time and

order of the appearance of ossification centers in the bones of the skull. Before the time of birth, all the bones except the ethmoid begin to ossify.

The first indication of ossification in the skull is found in the mandible at 12 days.

Fig. 4. Average ossification in the skull.

The mandible is removed. Explanation of the abbreviations is given in Fig. 3.

At 12 days and 12 hours, the maxilla and the premaxilla begin to ossify. In a few specimens, the former seems to be ossified earlier than the latter. At 12 days and 15 hours, the exoccipital, the basisphenoid, the parietal, the frontal, the squamosal, and the palatine begin to ossify approximately at the

same time. At 13 days, ossification centers of the basioccipital, the nasal and the vomer appear.

The appearance of the center in the vomer seemed to be delayed in a few specimens. The centers of the alisphenoid and the supraoccipital begin to appear at 13 days and 3 hours.

Fig. 5 Comparison of the rate and order of appearance of the ossification centers appearing in the rat, the mouse, and the hamster during the gestation period.

2. The vertebrae and the appendages

At 13 days and 15 hours, the tympanic, the interparietal and the zygomatic begin to ossify. The tympanic ossifies a little earlier than the others. The presphenoid ossifies at 14 days and 5 hours, and the basihyal and the lacrimal at 14 days and 15 hours. The ossification of the orbitosphenoid, which is the last of all the skull bones to ossify before birth, begins at 15 days. Fig. 3 and fig. 4 show the conditions of ossification at five different prenatal stages.

Discussion

Fig. 5 shows the rate and order of appearance of ossification centers in the rat [13], the mouse [8] and the golden hamster (present study). The gestation periods of these animals are 22.8 days for the rat, 20 days for the mouse, and 15.6 days for the hamster [9]. Gestation time of appearance of each ossification center was illustrated as a percent of the total gestation period in each animal. It is apparent from fig. 5 that in the hamster the ossification in the vertebrae, the appendages and the skull begins at a relatively later stage of gestation than that in the mouse and the rat as a result of the shorter gestation period in this animal. This is in accord with the view of Bruce [4] who studied the time and order of appearance of ossification centers in the rabbit skull, and compared the data with those of the man, the rat and the mouse. He concluded that the longer the gestation period, the earlier the relative stage of gestation when ossification centers appear, and that the animal of a shorter gestation period would be born with relatively immature skeletons.

As shown in fig. 5, in the vertebrae and the appendages, the appearance of each ossification center is almost in the same order among these three animals. In the skull, however, the order of ossification is not re-

gular in the nasal, the alisphenoid, the zygomaticum, the interparietal and the tympanic regions. Beatty et al. [2] made a study concerning the appearance of ossification centers in the hamster, in which materials were obtained during intervals of one day. The order of ossification appears to be quite indefinite. The time of appearance of ossification centers of each skeleton in our data is roughly 10 to 24 hours earlier than that of Beatty et al.

The body weight of the fetus of the hamster increases rapidly from the 13th day in gestation [9]. Ossification begins a day before the rapid increase of the body weight, which shows that the formation of the skeleton prepares for the rapid body growth. The newborn hamster represents a relatively younger skeletal age than the rat and the mouse especially in the manus and pes. In the rat and the mouse, more centers are present at birth than the hamster in the manus and pes [8, 13]. The prenatal growth of the hamster is indeed "explosive", but its skeletal system is still immature.

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ハムスターの骨化点出現時期と順序

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ゴールデンハムスター（クリーム系）の正常発育階程を求める目的で、411例の胎児を用い、全身骨格の骨化点出現時期と順序を調べた。

1. 最初の骨化点は11日15時間に鎖骨に現われ、以後順次各骨に骨化点が出現するが、その順序は頭蓋骨の一部を除いてラット、マウスのそれらと一致する点が多かった。

2. 骨化点の出現時期は鎖骨など非常に早いものを除いて、妊娠期間を100とした場合のほぼ80%からは

じまっている。これはラットの71.5%、マウスの75%よりも出現時期がさらに妊娠末期に片寄っていることを意味し、一般に妊娠期間の長いものほど骨格の骨化は妊娠の比較的早期に行われるという説を裏づけるものである。

3. ハムスター胎児の体重は妊娠13日から急激に増加するが、骨化は12日前後からはじまる。

4. 出生時の骨格はラット、マウスよりやや未熟な状態にあると考えられる結果を得た。