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TIME AND ORDER OF APPEARANCE OF OSSIFICATION CENTRES OF THE RAT SKELETON

By

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ABSTRACT

The time and sequence of ossification pattern of the skeletal system of the *Rattus rattus* were studied in serial whole mounts prepared from Day 10 to Day 21.5 (day of delivery). In the neurocranium, each bone is represent by a single ossification centra. At birth, the neurocranium is a well formed miniature of that in the adult. Four stages can be distinguished in the ossification of the vertebral column. All ossification centres in the vertebrae were found to be present at birth. In the appendicular skeleton, the chondrogenesis and osteogenesis patterns proceed in proximo-distal sequence with postero--anterior direction. It is possible to map limb part giving rise to each skeletal element as it appears. The pelvic limb bones are ossified later than those of the pectoral limb. Sexual dimorphism during ossification is not pronouced.

INTRODUCTION

Various investigations of mesenchymal, chondral and osseous stages of the skeleton of embryonic rodents have been undertaken (Strong 1920; Dawes, 1930; Johnson, 1933; Dalgleish, 1964; Rajtova, 1968; Theiler, 1972; Sterba, 1976; Mohammed, 1979 and El-Sayad, 1982). Studies on the chondrogenesis and osteogenesis of different animal species have revealed the phylogenetic relationship of these animals (Sewertzff, 1908; Romer, 1956; Dalgleish, 1964 and El-Sayad, 1982). The present investigation describes the ossification of neurocranium, vertebral column and appendicular skeleton during the prenatal development of the albino rat *Rattus rattus.*

MATERIALS AND METHODS

A series of albino rat embryos, *Rattus rattus,* from Day nine to Day 21.5 (day of delivery) postgestation, was used in this work. Males were placed with virgin females in the evening and separated at nine O'clock next morning. Females were examined for the presence of copulation plugs to verify fertilization. Females were maintained at room temperature (25±2°C) and a photoperiod of 12 hr/day. The age of embryos was determined on the assumption that ovulation occurred at the mid-point of the previous dark period (Snell *et al.* ., 1940).

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To illustrate the chondrogenic and osteogenic rudiments of the skeleton, whole mounts of embryos were stained by methyl green (McCann, 1971) and followed by alizarin red S (Dawson, 1926). The preparations were, then, examined using sterioscopic microscope at a magnification of X30. At each age, the lengths of fore and hind limb rudiments of five embryos (a total of 60 embryos) were measured, using an eyepiece graticule to the nearest 50 μm. Measurements were made whenever part of the central limb mesenchyme appeared distinctly more heavily stained with methyl green than the surrounding soft tissue.

RESULTS

Neurocranium (Figs 1 - 5). The neurocranium pattern is visible at about Day 16, when each rudiment appears as a cartilaginous tissue separated from other rudiments by various distances. Starting from Day 17, the ossification centres become gradually visible as the bone rudiments are formed. Gradually, the rudiments acquire a difinite arrangement and peripheral configuration giving each of them a characteristic shape which is clearly observed on Day 19. As these rudiments grow, they approach each other forming a system of frameworks which appear to have a mutual effect on determining the configuration of all the skull bones. At birth the skull is a maniature of adult's.

Vertebral Column (Fig. 6). Four stages can be distinguished in the development of the vertebral column. Stage 1; at Day 10, all the mesodermal cells of the axial skeleton are phenotypically identical. Stage two; a Day 13.5, the presumptive vertebral regions are determined. Stage three; a Day 16, the pattern of the whole cartilaginous vertebral column is clear. Stage four; a Day 18, the vertebrae start to acquire an osseous appearance. However, the ossification time of the vertebral bodies and their neural arches varies from one region to another, the earliest being the first and second sacral vertebrae, the latest being the coccygeal vertebrae.

Appendicular Skeleton (Figs. 7-9). Development is essentially similar in the fore and hind limb buds. At first, both limbs stain homogeneously until Day 10 when the stylopod, zeugopod and autopod cartilaginous rudiments become apparent. These three segments are well demarcated by Day 13 when the limb rudiments achieve their final chondrogenic form. The onest of osteogensis occurs at about Day 16 and proceeds until birth, when only the terminal parts of three segments remain cartilaginous. Ossification in the pectoral and pelvic limbs proceede in a proximo-distal sequence, starting with the humerus and femur and ending with the phalangeal region. There is no sign of ossification in the carpal or tarsal region. At Day 16, ossification of the scapula begins in the middle and proceeds so that at birth it is completely ossified. Dermal clavicle is very clear at Day 16.

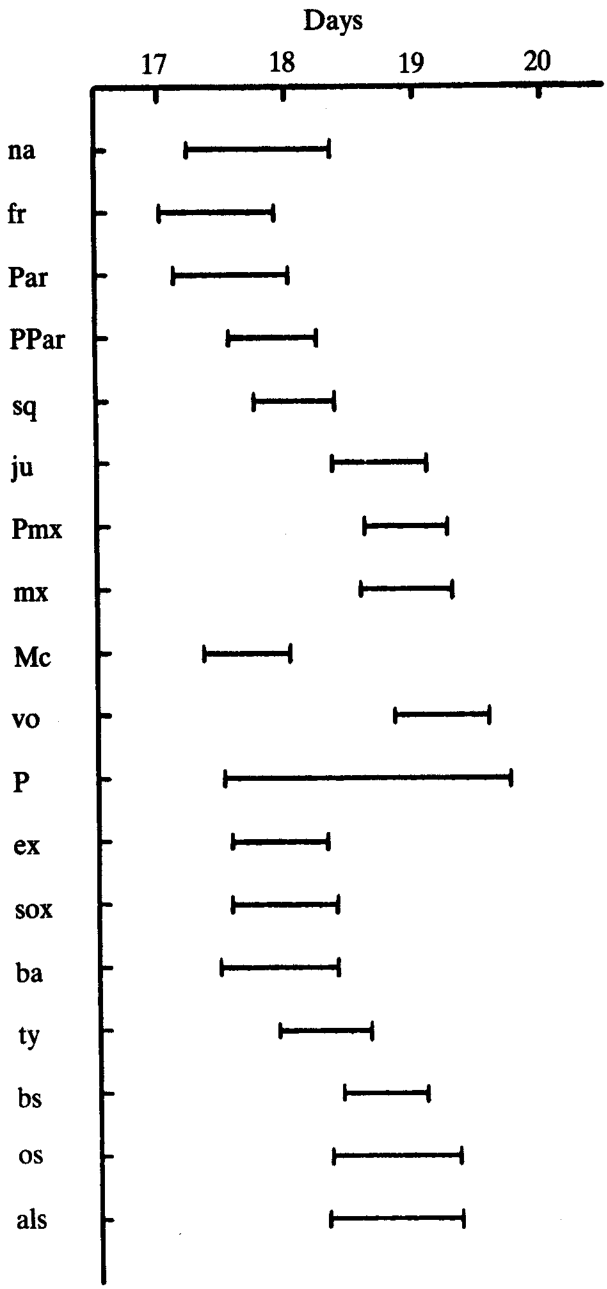
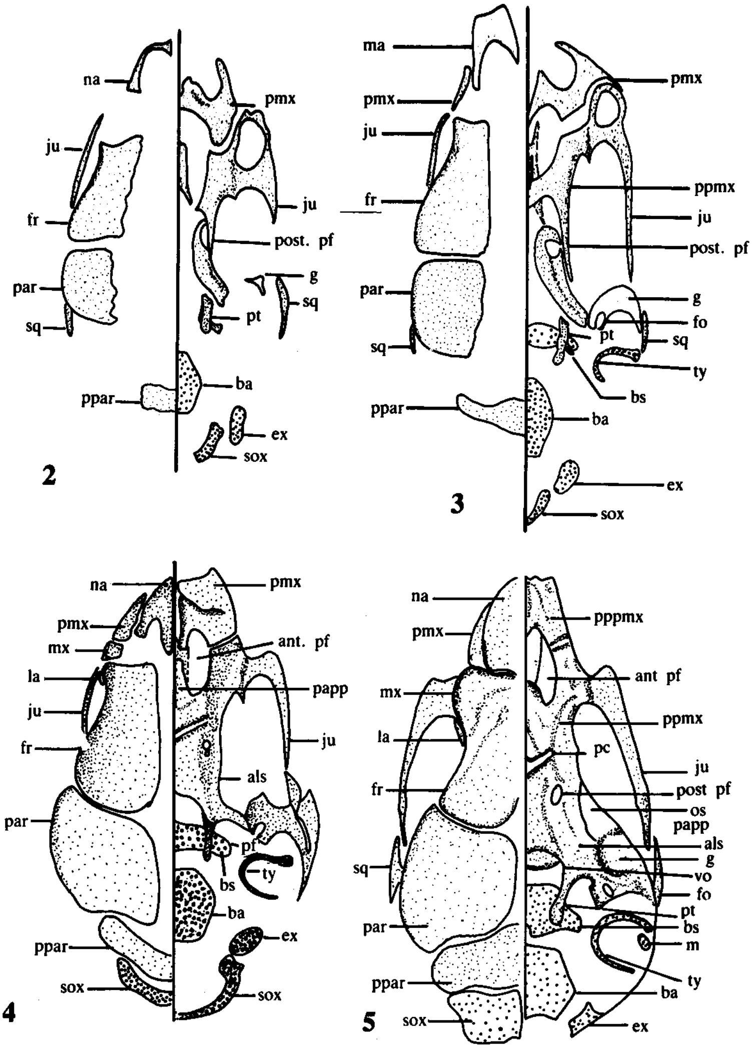


Figure 1. Appearance of ossification rudiments in the neurocranium of the rat, *Rattus rattus.*



Figures 2-5. Diagrammatic illustration of neurocranium rudiments of the rat, *Rattus rattus,* from dorsal (D) and Ventral (V) aspects during embryonic development. 1-Day 18.5, 2-Day 19, 3-Day 20 and 4-Day 21.5. als, alisphenoid; ant pf, anterior palatine foramen; ba, basioccipital; bs, basisphenoid; ex, exoccipital; fo, fenestra optia, fr, frontal; g, glenoid cavity; ju, jugal; la, lacrimal; m, malleus; mx, maxilla; na, nasal; os, orbitosphenoid; p, palatal group; papp, palatal process of palatine; par, parietal lamina; pc, palatine canal; pmx, premaxilla; post pf, posterior palatine foramen; ppar, postparietal; ppmx, palatine process of maxilla; pppmx, palatine process of premaxilla; pt, ptergoid; sox, supraoccipital; sq, squamosal; ty, tympanicus; vo, vomer.

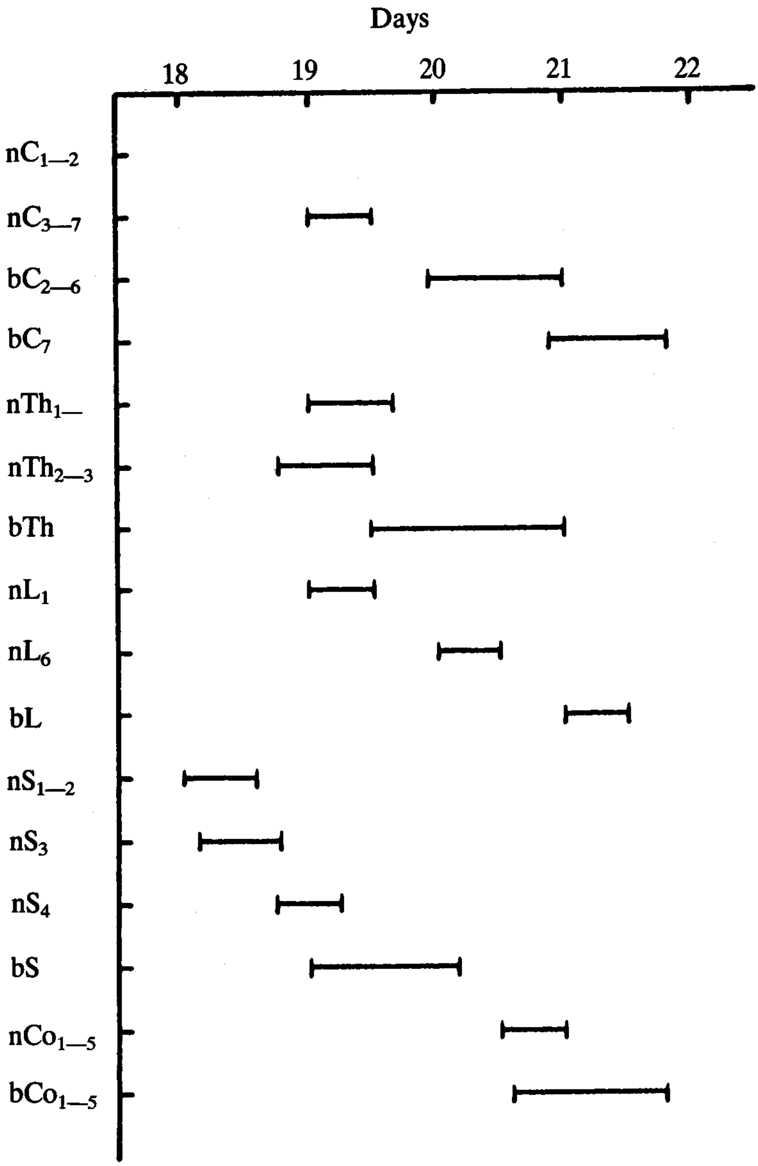


Figure 6. Appearance of ossification in the vertebral column. b, vertebral bodies; C, cervicales; Co, coccyheal; L, lumbales; n, neural arches; S, sacrales; Th, thoracicae.

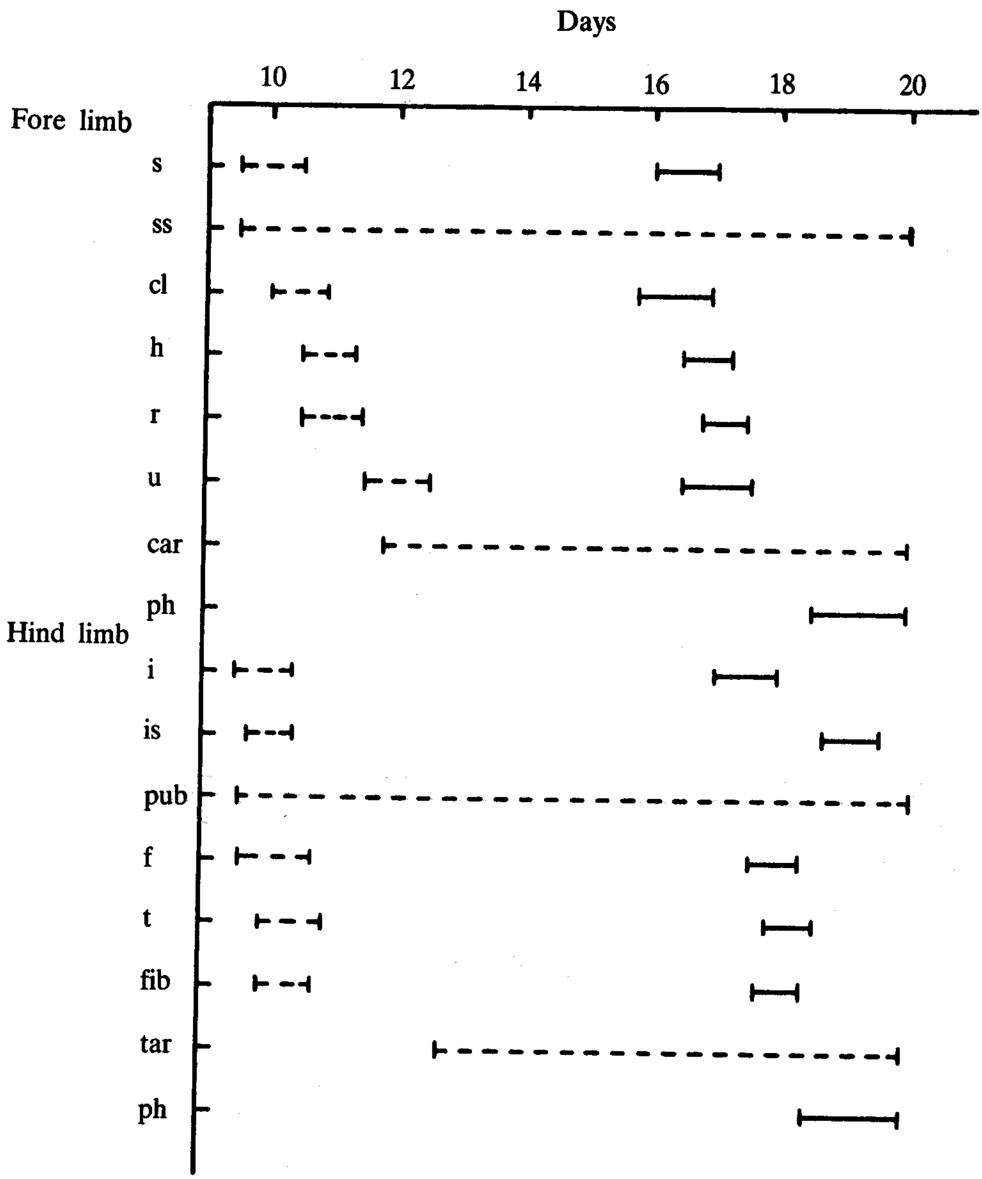


Figure 7. Appearance of cartilage rudiments and ossification of these rudiments in the fore and hind limbs of *R. rattus*. ca, carpals; cl. clavicle; fe, femur; fib, fibula; h, humerus; i, ilium; is, ischia; ph, phalanges; pub, pubis; r, radius; s, scapula; ss, suprscapula; t, tibia; ta, tarsus.

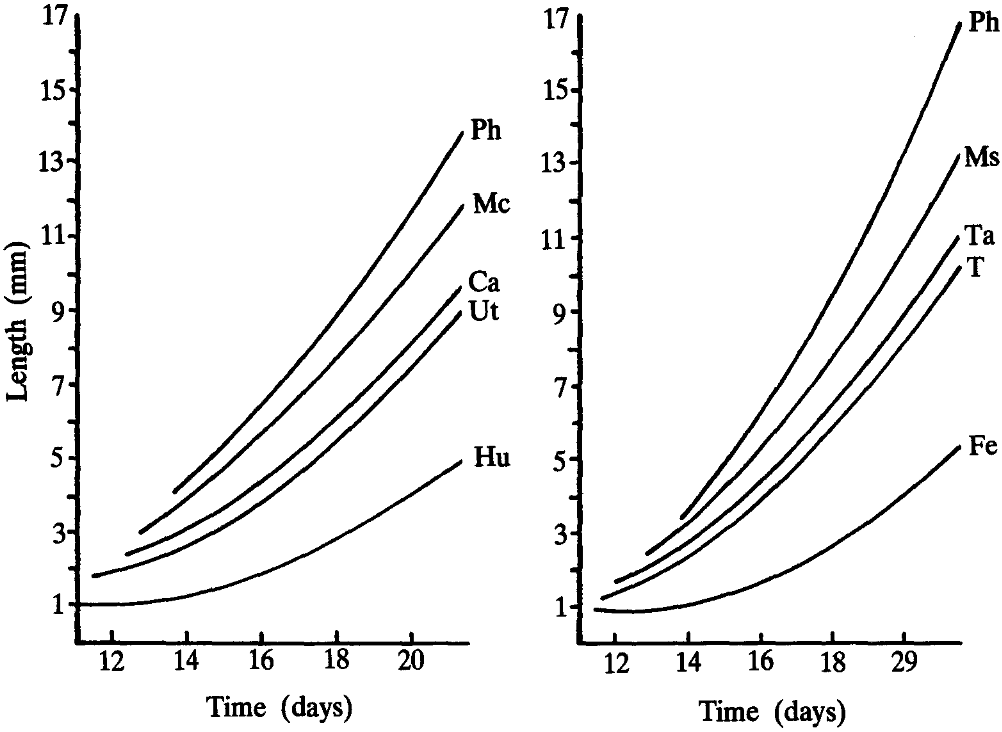


Figure 8. Growth curves for skeletal elements of fore and hind limbs.

For limb fate map, figure (9) illustrates the fore and hind limb growth, calculated as increase in length per original unit length per day. Although on Days 10-14, the rate of increase in length is fast, this rate is gradually decreasing becoming nearly constant starting with Day 16 for the stylopod and zeugopod and Day 17 for the autopod.

DISCUSSION

The overall pattern of the chondrification and ossification in the skeletal system of the embryos of the rat, *Rattus rattus,* is very similar to that described for other rodents by various authors (Strong, 1920; Dawes, 1930; Johanson, 1933; Dalgleish, 1964; Theiler, 1972; Mohammed, 1979 and El-Sayad, 1982). The homology increases among closely related species, and decreases among distantly related ones (Wright *et al.,* 1957; Rajtova, 1968 and Sterba, 1973). However, the beginning of ossification observed in the present study is different from that observed previously; difference may be partly explained by the degree of individual variation detected in the rat groups used in this study. Walker (1975) reported extensive variation among rats.

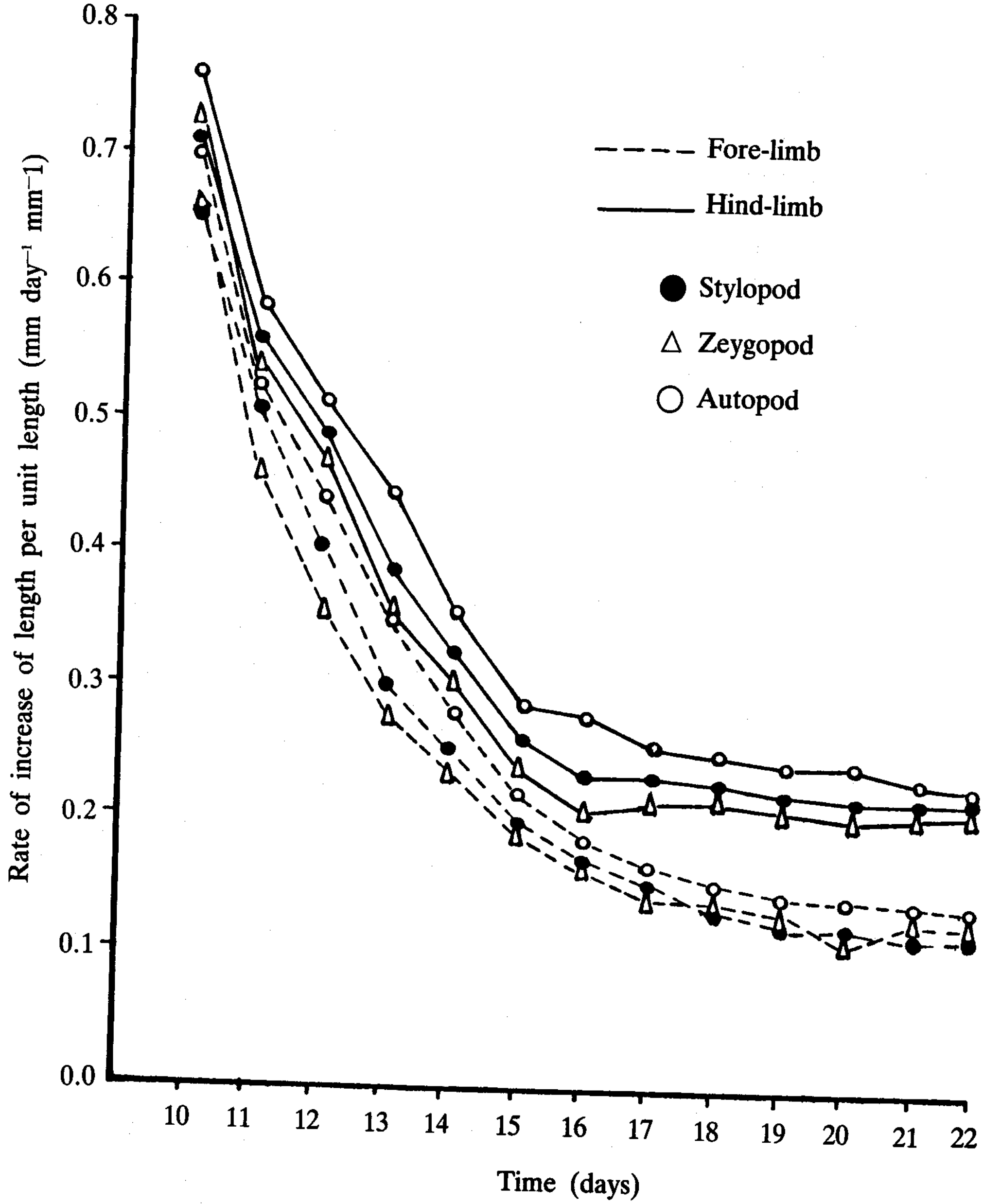


Figure 9. The rate of change of tissues at each level.

All bones of the neurocranium rudiments were represented by single centres of ossification. The presence of all centres at the time of birth agrees with most previous investigations (DeBeer, 1937; Theiler, 1972 and El-Sayad, 1982). There is a special distribution of ossification rudiments, and they are restricted to certain position with a definite surface extent and a characteristic shape. This distribution may be controlled by interaction of (1) the time and place of each rudiment, (2) the differentiation polarity i.e., the development of cartilaginous stages followed by osteogenous stages, and (3) the direction of the rudiment outgrowths to make proper arrangements of neurocranium rudiments.

In rat vertebrae, the ossification does not take place simultaneously, and is probably related to the presence of differentiating cartilage cells in different states. All ossification centres were found at birth, thus confirming the reports of Dawes (1930), Sterba (1973), Mohammed (1979) and El-Sayad (1982).

Following the ossification pattern of the fore and hind limb elements, it is evident that the limb ossification pattern undergows chondrogenesis pattern. The analogue has been described in chick (Saunders, 1948), lizard (Mathur and Goel, 1976), *Mus norvegicus albinus* (Strong, 1920), Guinea pig (Rajtova, 1968) and mole (Sterba, 1976). The ossification of the hind limb lags behind the ossification of the fore limb, and at birth no conspicuous difference was discerned. However, the limb elements become ossified at the same time of the axial skeleton, as ascertained in the house mouse (Theiler, 1972), Guinea pig (Rajtova, 1968) and mole (Sterba, 1976).

The changes in length of the fore and hind limb elements reflects more accurately what must be occuring at the cellular level, whether the change is caused by growth of cells, division of cells or production of intercellular matrix. These would suggest that the rat mesenchyme cells in the developing limb skeleton are assigned positional values in three-dimensional coordinate system. That pattern can be viewed in terms of the concept of positional information (Summerbell, 1976 and Wolpert, 1971); i.e. the positional value may determine the programme of chondrogenesis and developmental patterns of calcification for the rat limb skeleton.

From the present investigations, the morphogenesis of skeletal rudiments is the results of two factors: (1) rudiments develop becuse cells recognize their neighbours and know their positions within a skeleton tissue field and (2) synthesis of local morphogens which stimulate mesenchyme differentiation during cartilaginous stages and osteogenesis stages.

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زمن ونظام ظهور مراكز التعظم في هيكل الفأر

محمد بهجت حسين محمد و فوقية إبراهيم الصياد

ان دراسة التكوين الشكلي لقطع الجمجمة العصبية ، العمود الفقري ، الطرفين الأمامي والخلفي ، الحزامين الصدري والحوضي لأجنة الفأر الأبيض - عمر ٩ أيام حتى موعد الولادة - أوضحت تباين ظهور القطع الغضروفية وأزمنة تعظمها .

الجمجمة العصبية أظهرت تعظماً مفرداً لقطعها ، الفقرات أظهرت أزمنة مختلفة للتعظم داخل الفقرة الواحدة وبين الفقرات المختلفة ، الطرفان والحزامان تعظمها يتبع تكوين قطعهما الغضروفية . وقيست أطوال قطع الأطراف ومعدلات الزيادة لأطوالها .

وقد قورنت النتائج بالنماذج المتاحة في القوارض المختلفة .