THE UNIVERSITY OF ALBERTA

THE EMBRYOLOGICAL DEVELOPMENT OF THE HUMAN VERTEBRAL COLUMN

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE EMBRYOLOGICAL DEVELOPMENT OF THE HUMAN VERTEBRAL COLUMN submitted by DEBORAH JEAN SHANER in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE.

K.M. Bagwell

Supervisor

Date, December 13, 1985

DEDICATION

This work is dedicated to my parents, Ralph and Alberta Shaner and to my grandparents, Ralph and Jean Shaner and Jean Sjoberg, who always believed in my abilities and who gave me the encouragement to complete this project.

ABSTRACT

The vertebral columns of twenty-seven serially sectioned human embryos stained with hematoxylin and eosin were studied with a light microscope. The crown-rump lengths of the embryos ranged from 2 to 23.5 mm, representing a closely-graded series of embryos. The purpose of this study was to investigate the early development of the human vertebral column in its blastemal and cartilaginous stages. The development of the vertebral bodies, intervertebral disks, neural, costal and transverse processes throughout the entire extent of the vertebral column were studied. The main objective was to investigate the theory that the segments of the blastemal vertebral column undergo resegmentation to produce the cartilaginous vertebrae which are intersegmental with respect to the original segments. This study also investigated the nature of the intersclerotomal vessels and fissures as well as the intrasclerotomal fissures, all of which are controversial features in the literature dealing with human and non-human vertebral development. It was concluded that resegmentation of the blastemal segments does occur and that the intersclerotomal vessels and intrasclerotomal fissures are real entities in human embryos. The presence of the intersclerotomal fissures, however, could not be confirmed. A new theory of human vertebral development has been proposed in which the dense caudal sclerotome half and the less dense cranial sclerotome half of each segment change

position relative to the arteries and fissures: the light band surrounds the intersclerotomal artery and the dense band encompasses the intrasclerotomal fissure. The dark band is divided into two zones: a very dense cranial zone A and a less dense caudal zone B. Zone A forms the intervertebral disk while zone B chondrifies with the light band caudal to it, both contributing to the vertebral body. The neural and costal processes are outgrowths of zone B of the dense sclerotome band.

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LIST OF ABBREVIATIONS

A	zone A of the dark sclerotome band
a	aorta
al	allantois
at	atlas
ax	axis
B	zone B of the dark sclerotome band
b	band of cells
bv	blood vessel
C	cervical vertebra
cas	caudal sclerotome half
co	coelom
cp	costal process
crs	cranial sclerotome half
d	dermis
db	dark sclerotome band
dk	intervertebral disk
dm	dermatomyotomes
drg	dorsal root ganglion
ec	ectoderm
iap	inferior articulating process
in	intercostal nerve
isa	intersclerotomal artery
isb	intersclerotomal blood cells
isf	intrasclerotomal fissure
k	developing kidney
L	lumbar vertebra

lb	light sclerotome band
ls	lateral sclerotome
mb	myotomic bulge
mes	mesenchyme
ms	medial sclerotome
msb	mesenchymal band
mu	muscle tissue
my	myotome
nc	notochord
np	neural process
nt	neural tube
nvb	neurovascular bundle
oc	occiput
op	odontoid process
r	rib
rc	ramus communicans
sap	superior articulating process
scl	sclerotome cells
so	somite
son	suboccipital nerve
spn	spinal nerve
st	sympathetic trunk
S	sacral vertebra or sacrum
T	thoracic vertebra
tl	transverse ligament
v	vein
va	vertebral artery

vb vertebral body

I. INTRODUCTION

The purpose of this study was to examine the theory that the blastemal segments of the vertebral column resegment during human vertebral development and to establish the nature of the intersclerotomal vessels and fissures and the intrasclerotomal fissures in human embryos. Currently there are only seven theories of human vertebral development (Bardeen and Lewis, 1901; Bardeen, 1905a; Ehrenhaft, 1943; Wyburn, 1944; Sensenig, 1949, 1957; Peacock, 1951) and while there are differences between the theories, they all describe the process of resegmentation. Numerous studies of non-human tetrapods (for example Piiper (1928) on birds, Dawes (1930) and Sensenig (1943) on mice, Lawson (1966) on salamanders and Werner (1971) and Winchester and Bellairs (1977) on reptiles) also describe resegmentation of the segments during the formation of the vertebral column. However, recently several researchers studying non-human vertebral development (Wake and Lawson, 1973; Verbout, 1976, 1985; Dalgleish, 1985) have questioned the theory of resegmentation and have denied that it occurs during vertebral development. Wake and Lawson (1973) and Dalgleish (1985) have confined their conclusions to the species they studied. However, Verbout (1985) has concluded that resegmentation does not occur in any amniote species, including humans. Therefore, the present study was undertaken in an attempt to document the blastemal and cartilaginous stages of vertebral development and to examine

any evidence for or against resegmentation.

There is a great deal of confusion in the literature regarding the presence of the intersclerotomal vessels and fissures and the intrasclerotomal fissures. Reports of these features are highly variable in the literature on human and non-human tetrapod vertebral development, but it indicates that these vessels and fissures play an important role in this process. Therefore, human embryos were examined for the presence or absence of these features.

Human embryos were chosen for this study as there are few studies which have examined such embryos. There was also a need to reconcile the present theory of vertebral development with human congenital vertebral anomalies of formation as this has not been attempted in any detail by any of the researchers.

II. REVIEW OF THE LITERATURE

A. Techniques of Serial Reconstruction of Material Sectioned for Light Microscopy

Three basic types of reconstruction techniques for serial sections cut for light microscopy have been developed: graphical reconstruction, solid model reconstruction and serial section cinematography. The success of these models depends upon the condition of the original sections (Gaunt and Gaunt, 1978). If they are poor, this will be reflected in the reconstruction. Therefore, it is necessary to review the problem areas within the sections themselves.

Considerations in Histological Preparation and Sectioning

The first concern in the histological preparation of embryonic materials is the type of fixative used and its effects on the tissues. Patten and Philpott (1921) compared the shrinkage caused by six fixatives: Zenker's, 10% formalin, formol-alcohol, Orth, Tellyesnicky and Bouin's fluid on pig embryos of various crown-rump lengths (CRL). They also looked at the effects of dehydration and paraffin infiltration on these embryos. They concluded that the total shrinkage produced in embryos fixed in Zenker's, Orth and Tellyesnicky was approximately 25%, in 10% formalin and Bouin's it was about 20% and in those fixed in formol-alcohol it was 11%.

After fixation and embedding, consideration should be given to producing reference marks if reconstruction is anticipated. Reference marks are any impression which is introduced to the block prior to sectioning which maintains a constant relationship to the sections or material under investigation. They are used in serial reconstructions to align the sections or reconstructions. Three types of reference markers have been used: surface, included and photographic references.

In making surface references, the outer surfaces of the paraffin block were modified by trimming, marking, or painting. This was done prior to, or just after, sectioning. Early researchers (Alexander, 1877; Keibel, 1894; Strasser, 1887a, b, cited in Gaunt and Gaunt, 1978) tried to paint the outer edges of the block, but found that the pigment did not adhere well without first placing grooves on the surface of the block. There were other problems associated with the type of paint: it had to be resistant to the solvents used in processing the sections and not damage the surface of the wax block (Gaunt and Gaunt, 1978).

Included references were an alternative to surface references. They were materials incorporated into the wax block before sectioning which maintained a constant relationship to the tissue being sectioned. Liver and brain tissue (Davies, 1929; Gaunt and Gaunt, 1978) and chive leaves (Sita Lumsden, personal communication, cited in Gaunt and Gaunt, 1978) have been used for included references.

Larger tissues necessitated different techniques and materials for implanting reference marks; rather than placing the tissue under study within a reference tissue, the references were implanted in the wax block. Pigmented holes (Eychlesheimer, 1892), grass stems (Gaunt and Gaunt, 1978), nerve fibers (Neumayer, 1907, cited in Gaunt and Gaunt, 1978; Burston and Thurley, 1957) and hens' egg membranes (Barnett and Maxwell, 1960) have all been used as included references.

Not all researchers used included or surface reference marks. Heard (1931) devised a method for producing references in photographs of the sections rather than in the sections themselves. This was accomplished by filming the surface of the block, prior to cutting each section, using a camera with notches set into the aperture. The photographs were then used to guide the alignment of the reconstructed sections.

In cases where reference marks were not present in the sections the best fit method of alignment was widely used (for example: Wilson, 1983; Zaw-Tun and Burdi, 1985). For the purposes of alignment, structures in addition to those under investigation were traced and aligned subjectively. Gaunt and Gaunt (1978) recommended that at least six additional features, sectioned horizontally through their long axis, be included for the best fit method.

Once the embryos have been suitably fixed and embedded another source of potential error and distortion lies in the

sectioning process. Gaunt and Gaunt (1978) recommended a microtome with a flat cutting action such as is found in sliding or rotary microtomes. Those which produced curvature in the sections, such as the Cambridge pattern rocking microtome, were not recommended when the sections were to be reconstructed due to the distortion in the sections. The sections should not be less than 13 μm thick, as sections thinner than this exhibited more distortion upon mounting (Gaunt and Gaunt, 1978).

Various techniques have also been devised to help orient the tissue block to the microtome blade so that the precise plane of sectioning was known. Strasser (1887a, b, cited in Gaunt and Gaunt, 1978) and Born and Peter (1898, cited in Gaunt and Gaunt, 1978) placed parallel grooves on the sides of the block. Long (1924) incorporated silk thread into the block and Gaunt and Gaunt (1978) utilized paper with pencilled-in lines to produce orientation marks on the block.

Serial Reconstruction Techniques

Methods of solid model and graphical reconstruction are briefly reviewed here. Other more elaborate forms, such as serial section cinematography and stereoscopic models, were not attempted in this study and have not been included. Ware and LoPresti (1975) and Gaunt and Gaunt (1978) should be consulted for information on these forms of reconstruction.

Solid Model Reconstruction

Although His in 1868 (cited in Gaunt and Gaunt, 1978) first published the results of a study involving models constructed of clay and wax, it was Born (1876, 1883, cited in Gaunt and Gaunt, 1978) who first developed the method of wax plate reconstruction. Researchers following Born used modelling media such as gelatin, blotting paper, plaster of Paris, tinfoil, cork, leather, glass, wood, plastics, cardboard, celluloid and metal as alternatives to wax plates. Wax plates, however, were the first medium to be used and many modifications have been made to Born's original method of wax plate production and model reconstruction. The majority of these changes involved making the wax plates more durable (Gaunt and Gaunt, 1978). Mark (1906-07), Lewis (1915), Pohlman (1919), Moore and Hayden (1963) and Sack (1966) used Born's method of wax plate reconstruction, but made modifications to the technique. All of these researchers, with the exception of Pohlman (1919), reduced the number of steps involved in the production of a solid model, presumably with a concomitant reduction in the amount of error as fewer copies were made. Pohlman (1919) replaced the turpentine used to adhere the tissue paper with more wax. Lewis (1915) took photographs of the sections and traced them onto the wax plates rather than tracing the sections onto paper and then retracing them with carbon paper onto the wax plates as Born had done (Pohlman, 1919). Moore and Hayden (1963) produced a mixture of talcum

powder and butyl or amyl ester-type lacquer which, when sprayed on the wax plates, made the surface conducive to direct tracing from projected sections. Sack (1966) used an apparatus which projected the sections through translucent wax plates. The outlines were cut directly from the plates without prior tracing. Pedler and Tilly (1966) used a pantograph which was modified so that a heated wire simultaneously traced and cut out the sections in polystyrene sheets. Decades before, Mark (1906-07) utilized a similar principal to cut tracings in wax plates: alterations were made to a Wheeler and Wilson sewing machine so that a heated wire replaced the needle. In more recent times many researchers, including Gaunt (1955), Moore and Hayden (1963) and Gaunt and Gaunt (1978), used commercially-produced paraffin wax plates, presumably to alleviate the difficulties of hand-producing the wax plates.

Many researchers felt that wax models were expensive, difficult to make (particularly the plates themselves) and were not durable (Gage, 1907; Miller, 1931; Green, 1937; Saunders, 1940). Wallin (1913) solved the problem of poor durability of the wax models by electroplating them. The others, however, used a completely different modelling medium to rectify these problems. Lewis (1915) constructed plaster of Paris models whereas du Nouy et al. (1927) preferred plasticene. Green (1937) advocated the use of wood-pulp board which had been immersed in molten wax and Pedler and Tilly (1966) created models of polystyrene. Gage

(1907) developed a widely used method of solid reconstruction using blotting paper which others (Schaeffer, 1911; Miller, 1931, 1932; Saunders, 1940) subsequently adopted. Schaeffer (1911), Miller (1931) and Saunders (1940) made some modifications to the technique. Miller (1931) found difficulties with cutting the sections from blotting paper with a sewing machine, but found that a Cutawl machine worked well. Miller (1931) and Saunders (1940) both experimented with different glues to adhere the papers together rather than using pins as did Gage (1907). Schaeffer (1911) produced blotting paper models which he then cut open to show the internal aspect of each structure.

Graphical Reconstruction

Graphical reconstructions have ranged from tracings on transparent sheets which, when aligned and illuminated, resulted in a three-dimensional serial model, to tracing all of the sections on one sheet of paper and adding shading to produce a three-dimensional illustration. Perspective in the form of foreshortening has also been introduced to the illustration to create the impression of three dimensions.

One difference between solid and graphical reconstructions was that solid models could be manually rotated to any position, whereas graphical models usually only represented one view of an object. If another view was desired, the model often had to be re-reconstructed. The one exception to this was contour drawings produced on the

computer. These models could be redrawn or rotated to any angle on the screen (Gaunt and Gaunt, 1978). One of the drawbacks to graphical models was noted by Pedler and Tilly (1966) who argued that contour drawings on clear sheets did not portray depth adequately for detailed studies and that solid models were needed for full understanding of tissues.

The production of graphical models on transparent sheets involved essentially the same steps as were entailed in solid model reconstruction. The main difference was that the outlines were not cut out, but were simply traced and aligned. Thus, the number of production steps was reduced. Streeter (1905) used transparent paper whereas Senior (1929) used sheets of celluloid and gelatin, although he found that the latter ripped easily. Osborn (1967) photographed the sections first and traced from these. Brown and Arnott (1971) commented that model-making had many disadvantages such as errors in tracing and the lack of subtle contrast. Therefore, they photographed the sections and printed them on DuPont Cronar Ortho-Litho Type S Sheet Film which produced a transparent image of the sections.

Although contour drawings were not three-dimensional, many researchers used them to illustrate their work. Krieg (1949), Bang and Bang (1957) and Potts (1966) made contour drawings by tracing all of the outlines onto a single sheet of paper. Krieg (1949) and Bang and Bang (1957) distinguished each tracing from the other by using different colors for the outlines.

Perspective graphical models are essentially contour drawings, but they introduce depth, or perspective, in the form of foreshortening to the illustration. Halpern (1953), Mitchell and Thaemert (1965) and Poritsky (1969) used a perspective grid to produce these illustrations. Barnett (1956) employed a microprojector and a cylindrical convex lens to project sections onto a board angled at 30° to create perspective models. Dixon and Howarth (1958) rephotographed inked photographs at an angle of 45° to produce perspective models.

Computer models have been produced in essentially the same fashion as other graphical models and they are much like contour models. The sections were projected onto a coordinate plotter and traced with a cursor. Projection of the sections onto the back of a screen so that no shadows are cast during the tracing has been recommended. The sections were aligned and separated based on the section thickness. The resulting model could then be displayed on the screen and any view produced (Gaunt and Gaunt, 1978). Ware and LoPresti (1975) regarded the use of computers as important for quantitative analysis of the sections and models. Many researchers have used computers to reconstruct and study the nervous systems of invertebrates as these are small (Gaunt and Gaunt, 1978): for example, Glaser and Van der Loos (1965) used a computer-microscope to study neurons; Garvey et al. (1972) utilized a PDP-7 computer and a television-microscope scanner to analyze dendrites, whereas

Wann, et al. (1973) used a Digital Equipment Corporation-12 computer and a Zeiss Universal microscope for the same purpose. Levinthal and Ware (1972) and Willey, et al. (1973) used computers for the purpose of three-dimensional reconstruction. Levinthal and Ware (1972) used a computer to reconstruct the ganglia of Asplanchna brightwelli and Daphnia magna, but did not describe the apparatus. Willey et al. (1973) used an IBM 360/65 and a Rand computer tablet to reconstruct the synaptic boutons of a cat.

The methods of reconstruction are varied and several techniques can be employed in a single study. The advantages of reconstructions lie in their three-dimensional portrayal of two-dimensional sections. The wide range of materials which can be employed, from wax plates to computers, make reconstructions accessible to all researchers. The main problems in modelling techniques lie in the quality of the sections and the technique of alignment.

B. Human Vertebral Development

Human vertebral development has been a topic of controversy for over a century. The material utilized may be a contributing factor to the controversy over vertebral development (Verbout, 1985). Some workers (Bardeen 1905a, b, 1908a; Ehrenhaft, 1943; Wyburn, 1944; Peacock, 1951) did not describe their materials in sufficient detail to be very useful. Another contributing factor to the controversy over human vertebral development is the method used to study this

problem, namely observation of serially sectioned human embryos under a light microscope. This method has distinct disadvantages, the main one being that observation of slides is largely subjective with a great deal of the interpretation left to the researcher. However, since experimental techniques such as somite transplantation are not available for use on humans, observation of serial sections under the microscope is the only viable method. Furthermore, the ill-defined boundaries of the vertebral components in the early stages make it difficult to apply methods of reconstruction to the sections. Only four researchers have utilized reconstruction techniques to aid in the understanding of human vertebral development (Bardeen and Lewis, 1901; Bardeen, 1905a, b; Wyburn 1944; Sensenig, 1949).

There are only seven papers based on human embryos with theories of vertebral development: Bardeen and Lewis (1901), Bardeen (1905a), Ehrenhaft (1943), Wyburn (1944), Sensenig (1949, 1957) and Peacock (1951). Other researchers such as Dandy (1910) and Atwell (1930) have only studied and described single embryos. The long timespan these papers cover has led to a variety of terms which are not consistent from paper to paper and an attempt has been made here to standardize the terminology as much as possible.

It is important to stress that the literature reviewed here is based on human embryos. The theories of human vertebral development as described by Bardeen and Lewis

(1901), Bardeen (1905a), Ehrenhaft (1943), Wyburn (1944), Sensenig (1949, 1957) and Peacock (1951) have been diagrammatically illustrated in figures 40 and 41.

It is generally accepted that the somites form from paraxial mesoderm. However, only Dandy (1910) and Davis (1923) stated this with respect to human embryos. It is interesting that the descriptions of the somites are so varied. Bardeen and Lewis (1901) stated that the somites (which were referred to as myotomes although the figures indicate that these were somites) were oval in transverse section. Johnson (1917) described the shape of the twenty-fourth somite as a cube with four walls (medial, lateral, dorsal and ventral) while Davis (1923) depicted the somites as wedges with one point of the wedge flattened. Davis (1923) described the same borders as Johnson (1917), but also included cephalic and caudal borders which were seen to converge. Sensenig (1949) depicted only three walls in the somites: medial, lateral and ventral. The differing descriptions are due, at least in part, to whether the researchers described the somite boundaries in two or three dimensions. There may also be real differences in their morphology at different stages and in different regions of the vertebral column which would contribute to the varying descriptions.

Many researchers noted the presence of myocoeles within the somites (Bardeen and Lewis, 1901; Dandy, 1910; Davis, 1923; Atwell, 1930; Heuser, 1930; Sensenig, 1949, 1957;

Peacock, 1951) containing cells (Johnson, 1917; Sensenig, 1949). None denied the presence of myocoeles in the somites. Johnson (1917) stated that the cells in the myocoeles and those of the ventral walls and ventral part of the medial walls formed the sclerotomes. Bardeen and Lewis (1901) thought that the sclerotome cells were derived from the ventromedial aspect of the somites. Sensenig (1949) observed that only the ventral and medial walls of the somite provided the cells (found in the myocoele) which formed the mesenchymal vertebral column. Davis (1923), however, observed that all but the dorsal aspect of the medial boundaries and the dorsal walls provided cells for the vertebral column. Peacock (1951) observed that the cells from the medial aspect of the somite were involved in vertebral formation. Others (Sensenig, 1957; O'Rahilly and Meyer, 1979) stated only that cells of the somite contributed to the development of the vertebral column. It was not clear how these individuals determined which part of the somite contributed to the vertebral column from static, two-dimensional sections.

Various boundaries of the somites broke down to release the cells which contributed to the vertebrae: the ventral walls and the ventral aspect of the medial walls (Johnson, 1917), the medial wall (Peacock, 1951), and the ventral and ventromedial walls (Sensenig, 1949). These discrepancies may be attributed to the varying descriptions, or lack thereof, of the surfaces of the somite. Ehrenhaft (1943) did not

describe where the cells which formed the vertebral column originated; he simply referred to "mesenchyme".

There were several interpretations of the early dispersion of the cells from the somite to the area around the notochord. (Bardeen and Lewis (1901) and Wyburn (1944) did not detail the mechanism of dispersal). In one interpretation by Bardeen (1905a) and Ehrenhaft (1943) the cells released from the somite formed sclerotomes which retained their segmental nature by the presence of intersclerotomal vessels bordering the somites and their products. The sclerotomes were divided into cranial and caudal halves, or sclerotomites, by intrasclerotomal fissures. The caudal sclerotomite or scleromere became more dense and gave rise to three pairs of processes: the neural, costal and chordal processes. The chordal processes extended medially to the notochord, the neural processes extended dorsally around the neural tube and the costal processes extended ventrally or ventrolaterally. Johnson (1917) and Davis (1923), who each studied one embryo, observed only aortic and notochordal processes from the sclerotomes which extended to the area of the aorta and notochord respectively. Sensenig (1949, 1957) and Peacock (1951) did not report any processes, but only the migration of the sclerotome cells to the region of the notochord.

There is some confusion in the literature regarding the definition of the sclerotome. Sensenig (1949: 23) was the only one to define this term as the "...axial mesenchyme

between two adjacent intersegmental vessels...". Bardeen (1905a) and Ehrenhaft (1943) used the term apparently to describe the cells adjacent to the open somite whose caudal half possessed the various processes. Wyburn (1944) and Peacock (1951), on the other hand, used the term to describe the cells lateral to the notochord. Bardeen and Lewis (1901) do not refer to sclerotomes, but used the term "axial segments" which they did not define.

Intersclerotomal vessels are the center of considerable controversy in the literature dealing with human vertebral studies. They were described by Bardeen and Lewis (1901), Bardeen (1905a), Ehrenhaft (1943), Wyburn (1944), Sensenig (1949, 1957), Peacock (1951) and O'Rahilly and Meyer (1979) with only some researchers stating that they were branches from the aorta (Bardeen and Lewis, 1901; Ehrenhaft, 1943; O'Rahilly and Meyer, 1979). They were variously reported to mark the cranio-caudal limits of the somites (Bardeen and Lewis, 1901; Peacock, 1951) or the sclerotomes (Bardeen, 1905a; Ehrenhaft, 1943). (Bardeen and Lewis (1901) referred to myotomes, but the diagrams clearly indicate that these were somites). Sensenig described the vessels as forming incomplete boundaries of the sclerotomes (1949) or somites (1957). Sensenig (1949) was also the only researcher to have reported the presence of intersclerotomal fissures which arose prior to the vessels and which the vessels came to occupy. O'Rahilly and Meyer (1979) simply stated that the sclerotome around the notchord was segmented by vessels.

Wyburn (1944) did not make the position of the vessels clear.

Another feature of considerable controversy in the literature is the presence and morphology of the intrasclerotomal fissures. They were described by Bardeen (1905a), Ehrenhaft (1943), Wyburn (1944), Sensenig (1949, 1957), Peacock (1951) and O'Rahilly and Meyer (1979). Others, such as Bardeen and Lewis (1901) have neither confirmed nor denied the presence of these fissures. Bardeen (1905a) and O'Rahilly and Meyer (1979) noted the presence of the fissures, but did not describe them. Ehrenhaft (1943) and Wyburn (1944) described the fissures as forming the dividing line between the dense and loose sclerotome halves. Peacock (1951) reported the presence of the intrasclerotomal fissures within the group of cells migrating toward the notochord. Sensenig stated that the fissure divided each sclerotome (1949) or somite (1957) into halves, but did not form a complete division.

There also appears to be little agreement as to the fate of the sclerotomal processes or cells migrating from the somite. Bardeen and Lewis (1901) were vague, stating only that the caudal third of each sclerotome was dense and represented the intervertebral disk as well as the neural and costal processes. Bardeen (1905a) observed that the chordal processes joined those opposite to them around the notochord, thus forming the primitive disks. The cranial halves of the sclerotomes provided interdiscal membranes

which loosely filled the areas between the primitive disks. The primitive disks lost cells to the cranial half of the next segment and the primitive disks were then reinforced by the cranial sclerotome half of the same segment. Ehrenhaft (1943) stated that the scleromere gave off a process which migrated to the notochord as the primitive vertebral body while the caudal half of the scleromere lost cells to the next adjacent segment. The rarified area was then repopulated with mesenchymal cells whose origin Ehrenhaft (1943) did not detail. The dense zone was also reported to be cranially "displaced", which Ehrenhaft (1943: 282) attributed to the "...intersegmental vessels and the increased nutrition which is available to the cells closest to those arteries". The dense zones were called the primitive intervertebral disks and the looser areas the anlage of the true vertebral bodies. Like Bardeen (1905a), Ehrenhaft (1943) described an interdiscal membrane, but concluded that it originated from the primitive intervertebral disks and surrounded the anlage of the vertebral bodies. Wyburn (1944) reported that the caudal half of the sclerotome produced processes which joined at the notochord while the areas between these dense processes appeared to be populated by tissue from the cranial half of the sclerotome. The dense material around the notochord was reinforced by cells from the cranial half of the sclerotome; these two areas then formed the perichordal disks. Sensenig (1949, 1957) stated that the cells which migrated to the

notochord were initially more dense in the center of each segment than in the region of the intersclerotomal fissure. The cells were also more condensed laterally towards the myotome than medially around the notochord. In more advanced embryos, the upper two-thirds of the caudal sclerotome half and the lower one-third of the cranial half of each segment were more dense (Sensenig, 1949). In another study by Sensenig (1957) it was reported simply that the caudal sclerotome half was more dense than the cranial sclerotome half. Peacock (1951) described dense tissue above and below the intrasclerotomal fissure in the same proportions as did Sensenig (1949) and stated that it formed the intervertebral disks. In less developed embryos, the dense tissue was located below the intrasclerotomal fissure and looser tissue above the fissure. Although all of these researchers reported the presence of differing densities, none had quantified the observations by counting the number of cells in the light and dark areas, measuring the size of the cells in the two areas or measuring the intensity of the stain in these areas. Other than that of Bardeen and Lewis (1901) and Sensenig (1957), all of these descriptions indicate that the dense areas surrounded the intrasclerotomal fissures.

Both Wyburn (1944) and Sensenig (1949) reported the presence of differing densities within the perichordal disks: a very dense zone C and two less dense zones, A and B, cranial and caudal to zone C respectively. The remaining less dense zone was named the primary centrum by Sensenig

(1949) and zone D by Wyburn (1944). No others have reported this phenomenon in human embryos. Unfortunately, the photographs showing these three areas are very small and do not clearly indicate these zones.

Several researchers described the early development of a sheath around the notochord. Bardeen (1905a) reported that the cells of the axial mesenchyme aggregated around the notochord with their long axes parallel it. Ehrenhaft (1943) described mesenchyme (origin unstated) around the notochord forming the notochordal sheath. Sensenig (1949, 1957) stated that the cells around the notochord were of sclerotomal origin. Peacock (1951) described two different formations around the notochord: several layers of mesodermal cells and an acellular sheath. The latter was termed the notochordal sheath and it went through changes at chondrification, becoming thin within the vertebrae and thick between them. Both Bardeen (1905a) and Ehrenhaft (1943) reported that the notochordal sheath extended ventrodorsally to divide the anlage of the vertebral body into halves. Initially it separated the centers of chondrification, but the sheath broke down shortly after the onset of chondrification. None of the researchers reported the notochordal sheath to have any role in the development of the vertebral column other than separation of the centers of chondrification of the vertebral body (Bardeen, 1905a; Ehrenhaft, 1943).

With chondrification, the definitive vertebrae and intervertebral disks emerged. Bardeen and Lewis (1901).

stated that part of the sclerotome (not involved in the formation of the neural and costal processes) developed into the intervertebral disk and the areas between them chondrified as the vertebral bodies. Bardeen (1905a) concluded that the vertebral bodies were derived from the less dense sclerotome between the primitive disks and the intervertebral disks from the primitive disks. Ehrenhaft (1943) stated that the vertebral body formed from chondrification of the lower area of the caudal sclerotome half which had initially become rarified and the cranial sclerotome half of the next segment. The intervertebral disk formed from the dense zone surrounding the intrasclerotomal fissure. Both Wyburn (1944) and Sensenig (1949) concluded that the vertebral bodies chondrified from the less dense areas and zone B of the perichordal disk above and zone A of the perichordal disk below. Zone C formed the intervertebral disk. Sensenig (1957) stated that the dense sclerotome half gave rise to the intervertebral disk and the cranial and caudal aspects of adjacent vertebral bodies, while the less dense areas also gave rise to part of the vertebral body. Peacock (1951) did not discuss the origin of the vertebral bodies, but did observe that the intervertebral disks originated from the area of dense cells around the intrasclerotomal fissure. He also reported that the cranial and caudal aspects of the disk formed "cartilage plates" (Peacock, 1951: 263), but did not elaborate on these in any detail.

The costal and neural processes were reported to be projections from the caudal sclerotome half by most researchers (Bardeen 1905a; Ehrenhaft, 1943; Wyburn, 1944; O'Rahilly and Meyer, 1979) or lower one-third (Bardeen and Lewis, 1901) of the sclerotome. Sensenig (1949), however, stated that both processes were intersegmental in origin, with the caudal half of one segment and the cranial half of the next segment contributing to the neural and costal processes. In 1957 Sensenig came to the same conclusion regarding the neural processes, but did not describe the costal ones in any detail. Peacock (1951) did not discuss the neural or costal processes in any detail.

The neural and costal processes were reported by Bardeen (1905a) to be forced caudally between the myotomes by the myotomic bulge opposite the intrasclerotomal fissure, whereas Sensenig (1949, 1957) stated that the neural processes were originally positioned between the dorsal root ganglia and the costal processes between adjacent myotomes. Wyburn (1944) explained that the costal processes were at a higher level than the neural processes and that the amount of chondrification of the periphery of the perichordal disks succeeded in bringing the costal processes to their adult position.

The neural and costal processes were joined by a variety of mesenchymal bands. Bardeen and Lewis (1901) observed a (unnamed) band of cells between the processes of adjacent segments. Bardeen (1905a) and Ehrenhaft (1943)

reported interdorsal membranes, deriving from the cranial half of the sclerotomes, between neural processes and Ehrenhaft (1943) also observed the same type of membrane between the costal processes. Sensenig (1949) described the membrana reuniens dorsalis which, unlike the other membranes, joined the neural processes of the same segment. Peacock (1951) described a membrane, the hypochordal bow, originating from the intervertebral disk which joined the head of the rib in the thoracic region.

In studies of the entire vertebral column, such as that by Bardeen and Lewis (1901), it was not always made clear whether costal processes were present in all regions of the vertebral column. Bardeen (1905a) and Wyburn (1944), who studied only the thoracic region, described costal processes which developed into ribs. Bardeen reported the presence of costal processes in the cervical vertebrae (1908a, b) as well as in the lumbar, sacral and first coccygeal vertebrae (1905b, 1908b). All of the costal processes in these regions were seen to fuse with the transverse processes during chondrification (Bardeen, 1905b, 1908a, b), although in the cervical region only the distal tips of the processes fused (Bardeen, 1908a). In the sacral region, the costal processes also fused together to form the lateral sacral plate (Bardeen, 1905b, 1908b). Wyburn (1944) and Sensenig (1957) both mentioned costal processes in the cervical region, but neither described their fate. Sensenig (1949) described rib rudiments, but did not make it clear whether or not they

were present in any vertebrae other than the thoracic.

Few researchers speculated on the origins of the transverse and articular processes. Bardeen and Lewis (1901) stated that the transverse processes were derivatives of the dense caudal third of each sclerotome. Bardeen suggested that in the cervical region the transverse and articular processes arose from the neural processes (1908a), as did the transverse processes of the thoracic region (1905a) and the transverse and articular processes of the lumbar, sacral and first two coccygeal vertebrae (1905b). Bardeen (1905b) also noted that in the lumbar region the superior articulating processes developed at a faster rate than did the inferior processes. Sensenig's diagram (1949: Fig. 1) suggests that the transverse processes of the thoracic region were derivatives of the caudal sclerotome halves. It also indicates that the superior articular processes were derived from the caudal sclerotome half, while the majority of the inferior processes were formed from the cranial sclerotome half of the next segment. The tips of both the superior and inferior articulating processes were derivatives of the cranial sclerotome half.

The odontoid process is a specialized feature of the second cervical vertebra and few researchers have studied its development. Bardeen (1908a) observed that the body of the atlas joined that of the axis by the conversion of the intervertebral disk between them into cartilage. Chondrification of the disk began laterally and proceeded to

the center of the disk. Both Cave (1938) and Sensenig (1957) considered the odontoid process to be composed of two parts of different origins. Cave (1938) did not state the origin of the basal part of the odontoid, but described the apical portion as the body of the proatlas. The arch of the proatlas joined with the neural arch of the first cervical vertebra. According to Sensenig (1957) the cranial half of the first vertebral sclerotome formed the proatlas, which had a body and a neural arch. The body of the atlas formed the majority of the odontoid process while the body of the proatlas contributed to its tip. The neural arch of the proatlas joined the occipital condyles.

All of the researchers who formulated a theory of human vertebral development (see above) invoked the mechanism of resegmentation in the blastemal stages of vertebral development, but the actual processes of vertebral development are varied. Bardeen (1905a) and Ehrenhaft (1943) appear to have described a similar process, as have Wyburn (1944) and Sensenig (1949). The reasons for the differing theories are not clear, although it is probable that differing interpretations may account for many of these discrepancies.

C. Non-human Vertebral Development

The vertebral column of tetrapods has been studied extensively by many researchers. The importance of non-human vertebral development to the study of the human vertebral

column has been elucidated by Williams (1959), Jarvik (1980) and Verbout (1985). Williams (1959) and Verbout (1985) have stated that vertebral development is essentially the same in all amniotes and that researchers have interpreted the same process differently. Jarvik (1980) stated that all vertebrates have the same vertebral development, but specializations result in some differences in this process.

Several of the most important papers from each class of tetrapod (amphibians, reptiles, birds and non-human mammals) have been reviewed. Only typical vertebral development has been discussed and specialized vertebrae such as the atlas and axis have been omitted. Mookerjee (1930), Lawson (1966) and Wake and Lawson (1973) have each studied amphibian vertebral development in Triton vulgaris (salamander), Hypogeophis rostratus (apodian) and Eurycea bislineata (salamander), respectively. Reptiles have been investigated by several people. Howes and Swinnerton (1901) studied vertebral development in Sphenodon punctatus, Werner (1971) studied three geckos, Ptyodactylus hasselquistii, Sphaerodactylus argus and Hemidactylus turcicus, while Winchester and Bellairs (1977) looked at two lizards, Lacerta vivipara and Anguis fragilis and one species of snake, Natrix natrix. Bird vertebral development was studied by Piiper (1928) in Larus canus (gull) and Struthio australis (ostrich) and by Williams (1942) in Gallus domesticus (white leghorn chick). Two types of mammals have been investigated: mice and sheep. Dawes (1930) detailed the

vertebral development of Mus musculus (common white mouse), Sensenig (1943) studied Peromyscus maniculatus (deer mouse) and Dalgleish (1985) studied DBA mice. Verbout (1985) was the only researcher to develop a theory of sheep vertebral development in Ovis aries. The entire vertebral column was studied in most cases, with the exceptions of Winchester and Bellairs (1977) who only looked at the mid-trunk and mid-tail vertebrae, Piiper (1928) and Dawes (1930) who studied the cervical and thoracic vertebrae, Williams (1942) who observed the cervicals and Dalgleish (1985) who studied only the thoracic vertebrae.

Serial sections were studied by the researchers although the number of embryos varied considerably. The sections were often supplemented with whole mounted specimens and skeletons. Only Howes and Swinnerton (1901) and Dawes (1930) utilized wax plate and graphical reconstructions, respectively, to supplement their observations.

There is a wide variety of terminology in the literature, with some researchers using the arcualia terminology, while others rejected this in favor of the sclerotome terminology. In most cases, these terms are interchangeable and therefore have been standardized as much as possible. The basalia (basidorsals and basiventrals) arose from the dorsal and ventral aspects of the caudal sclerotome half respectively and the interbasalia (interdorsals and interventrals) were derivatives of the

dorsal and ventral aspects of the cranial sclerotome half respectively. Dawes (1930) differed slightly in his interpretation of the basidorsals; he stated that there was also a small contribution from the cranial sclerotome half. Mookerjee (1930) did not describe the presence of the interbasalia, but only discussed the basalia. Verbout's (1985) scheme is not easily categorized as he completely rejects the sclerotome terminology and has devised his own. The somitic mesenchyme is roughly equivalent to the sclerotome of others and is segmented by arteries at the myotomal junctions. However, he denied the presence of definitive sclerotome halves and preferred to use terminology such as "the caudal part of the segment".

None of the researchers detailed the shape of the somites or their breakdown. The presence of the intrasclerotomal fissures and intersclerotomal vessels and fissures are as controversial in non-human tetrapod development as they are in human studies. None of the researchers working on amphibian embryos reported the presence of intrasclerotomal fissures or intersclerotomal fissures and vessels (Mookerjee, 1930; Lawson, 1966; Wake and Lawson, 1973) nor did Howes and Swinnerton (1901) in their reptilian embryos. However, all other researchers did note the presence of all or some of these features. Sensenig (1943) and Werner (1971) did not note the presence of the intersclerotomal fissures while Winchester and Bellairs (1977) did not report the presence of vessels in the

intersclerotomal fissures. Dalgleish (1985) did not make it clear whether or not intrasclerotomal fissures were present. All others (Piiper, 1928; Dawes, 1930; Williams, 1942; Verbout, 1985) observed these three features. Only Dawes (1930) and Verbout (1985) specifically referred to the intersclerotomal vessels as arteries and Verbout (1985) stated that they were branches from the aorta. Dawes (1930) and Verbout (1985) were the only ones to observe the presence of veins, in addition to arteries, in the intersclerotomal fissures. Only Sensenig (1943) stated that the intersclerotomal blood vessels did not mark the exact boundaries of the sclerotomes and that both the blood vessels and the intrasclerotomal fissures only partially divided the sclerotomes. Dalgleish (1985) noted that the intersclerotomal fissures did not prevent the intermingling of the cells from adjacent sclerotomes, although he did not describe how this was determined. Both Dalgleish (1985) and Verbout (1985) felt that the intrasclerotomal fissures were artifactual.

There is some controversy over the extent of the vessels and fissures. Dawes (1930) and Werner (1971) both stated that the intrasclerotomal fissures entered the substance of the perichordal tube (see below) whereas Dalgleish (1985) indicated that these did not enter it. Winchester and Bellairs (1977) stated that neither the intersclerotomal nor intrasclerotomal fissures were located in the perichordal tube, while Piiper (1928) mentioned that

the intersclerotomal blood vessels and fissures did not enter the tube. No others commented on the precise extent of the fissures and vessels.

Almost all of the researchers who reported the presence of inter- and intrasclerotomal fissures also stated that the caudal sclerotome half was more dense than the cranial half (Piiper, 1928; Dawes, 1930; Werner, 1971; Winchester and Bellairs, 1977; Dalgleish, 1985). Williams (1942), Sensenig (1943) and Verbout (1985) are exceptions. In Williams' (1942) embryos, the most dense aspect was in the region of the intrasclerotomal fissure. Parts of both sclerotome halves were dense in Sensenig's (1943) embryos: in the cranial half the lateral and caudomedial aspects were dense, while all of the caudal half, other than a small caudomedial area, was dense. Verbout's (1985) terminology separated his findings from that of the others; he stated that the caudal aspect of each segment was more dense.

In general, the centra were described as having developed from the perichordal tube which was of somitic origin and which surrounded the notochord. The perichordal rings were described as sclerotomal structures by Piiper (1928), Mookerjee (1930), Dawes (1930), Williams (1942), Sensenig (1943), Lawson (1966), Werner (1971), Wake and Lawson (1973), Dalgleish (1985) and Verbout (1985). Of these researchers only Lawson (1966), Wake and Lawson (1973) and Winchester and Bellairs (1977) were not clear as to whether the rings were intrasegmental structures or not and Howes

and Swinnerton (1901) did not describe perichordal rings at all in their specimens. Piiper (1928), Dawes (1930), Williams (1942), Sensenig (1943), Lawson (1966), Wake and Lawson (1973), Dalgleish (1985) and Verbout (1985) stated that the cells between the perichordal rings were sclerotomal in origin. Mookerjee (1930) observed that cells other than those from the sclerotomes produced these vertebral regions, but did not make the origin of these cells clear. Howes and Swinnerton (1901), Werner (1971) and Winchester and Bellairs (1977) did not state the origin of the cells in the interspaces between the perichordal rings.

In more advanced tetrapods, the birds and mammals, the arcualia or sclerotomes also contributed to the vertebral centra. In amphibians (Mookerjee, 1930; Lawson, 1966; Wake and Lawson, 1973) and reptiles (Werner, 1971; Winchester and Bellairs, 1977) the intrasegmental perichordal rings and intersegmental vertebral regions alternated in the perichordal tube. In birds (Piiper, 1928; Williams, 1942) and mammals (Dawes, 1930; Sensenig, 1943; Dalgleish, 1985; Verbout, 1985) the sclerotome halves contributed to the perichordal tube to form the centra. Piiper (1928) and Williams (1942) both came to the conclusion that the prospondylous and opisthospondylous zones of the perichordal rings joined the vertebral regions to form the primary centra. The secondary or definitive centra were formed from the primary centra and parts of the adjacent arcualia. Dawes' (1930) and Sensenig's (1943) work on mice indicated

that bird and mouse vertebral development was essentially the same. Neither researcher placed much emphasis on the perichordal tube, but rather emphasized the sclerotomes. Dalgleish's (1985) and Verbout's (1985) findings were quite different from other researchers' results in bird and mammalian embryos. In Dalgleish's (1985) mice, the centra developed like those of amphibians and reptiles: from only the loose areas of the perichordal tube. Verbout's (1985) findings were different from those of others due to his terminology (see above). The loose areas of the perichordal tube, in addition to parts of the neural processes and ribs (the latter only in the cervical and lumbar regions) of the caudal half of the segments, formed the vertebral bodies.

The perichordal rings (the parts not involved in vertebral formation) formed the intervertebral disks, except in some of the amphibians and reptiles. In some amphibians (Mookerjee, 1930; Wake and Lawson, 1973) the perichordal rings contributed to the centra as articular surfaces, while in other amphibians (Lawson, 1966), reptiles (Howes and Swinnerton, 1901; Werner, 1971; Winchester and Bellairs, 1977), birds (Piiper, 1928; Williams, 1942) and mammals (Dawes, 1930; Sensenig, 1943; Dalgleish, 1985) the perichordal disks developed into intervertebral disks. In birds (Piiper, 1928; Williams, 1942) the definitive intervertebral disk had contributions from the arcualia and were ultimately composed of the middle zone of the perichordal ring (primary intervertebral body) and the

caudal aspect of the interdorsals. Williams (1942) was unsure of the contributions of the arcualia to the intervertebral disk and, as an alternative, proposed that migrating connective tissue cells might form the outer covering. Verbout (1985) also thought that the definitive intervertebral disk was a compound structure from the dense parts of the perichordal tube in addition to a small amount of the dense cranial somitic mesenchyme of the axial region.

There is general agreement among the theories of vertebral development that the caudal sclerotome half (basidorsal) contributed to the neural arches. (Lawson (1966) was the only researcher not to describe the neural processes in any detail). Some researchers thought that the neural processes were intersegmental structures, arising from the caudal sclerotome half and the subjacent cranial sclerotome half (Piiper, 1928; Williams, 1942; Winchester and Bellairs, 1977). Dawes (1930) considered the basidorsals to be composed of the caudal sclerotome half as well as a small part of the cranial sclerotome half of the same segment, resulting in intrasegmental neural arches. Sensenig (1943), Werner (1971) and Dalgleish (1985) were the only researchers to report that the neural arches were derivatives only of the caudal sclerotome halves. Verbout (1985) appeared to agree with these workers, although in his scheme the neural processes were derivatives of the dense arcocostal triangle, or the dense caudal area of each segment. Howes and Swinnerton (1901) and Wake and Lawson

(1973) described the neural arches as outgrowths of the skeletogenous sheath (perichordal tube) and prevertebral cells (whose origin was not discussed) respectively. Mookerjee's (1930) description of the development of the neural arches was more complicated and detailed than that of any other researcher. He postulated that the area between the basidorsals of two vertebrae was filled with fibrous tissue and had roofs of connective tissue in addition to the supradorsal connecting pieces. All of this tissue ossified, except for a small area between the successive basidorsals. Each basidorsal was joined to the fibrous tissue arches cranial and caudal to it, producing the neural processes.

There is also some controversy as to how the neural arches were joined dorsal to the neural tube. (Williams (1942), Werner (1971) Dalgleish (1985) and Verbout (1985) did not address this problem). There were two methods reported in the literature: a third separate element joined the processes (Piiper, 1928; Mookerjee, 1930; Sensenig, 1943) or the processes of one vertebra joined directly without the aid of any connecting pieces (Howes and Swinnerton, 1901; Wake and Lawson, 1973; Winchester and Bellairs, 1977).

There is also little agreement as to the origins of the articular processes. (Howes and Swinnerton (1901), Lawson (1966) and Dalgleish (1985) did not describe these features). Dawes (1930) and Sensenig (1943) stated that parts of the cranial sclerotome halves (dorsal-interdorsals)

formed the articulating processes exclusively. In Werner's (1971) reptiles the processes were compound structures arising from the caudal sclerotome half and from the the cranial sclerotome halves adjacent to it. Piiper (1928), Williams (1942) and Winchester and Bellairs (1977) agreed that the superior articulating processes were derived from the caudal sclerotome half and the inferior articulating processes from the cranial sclerotome half (dorsal-interdorsal). Wake and Lawson (1973) stated that the superior processes were cartilaginous rods (whose origin and development was not discussed) and the inferior processes were outgrowths of the neural arch in their specimens. Mookerjee (1930) stated that the dorsointervertebrals, (which were of sclerotomic origin and seemed to be homologous to the dorsal-interdorsals) produced the articular processes. Verbout (1985) did not give any details on the strand of tissue between the neural arches which formed the articular processes.

The ribs are another controversial feature in vertebral development. (Howes and Swinnerton (1901), Mookerjee (1930), Lawson (1966) and Winchester and Bellairs (1977) did not describe the origin and early development of the ribs). Piiper (1928), Dawes (1930), Williams (1942) and Werner (1971) stated that the ribs arose from the caudal sclerotome halves (basiventrals). Sensenig (1943) was the only researcher to describe the ribs as intersegmental structures, which were formed from both the cranial and

caudal sclerotome halves. Dalgleish did not discuss the ribs, but his figures 10, 11 and 12 (1985: 96) indicated that they were projections of the dense perichordal disks. Verbout (1985) described the ribs as extensions of the ventrolateral process of the arcocostal triangle.

The only researcher to fully describe the development of the chevron bones was Mookerjee (1930). They developed from the basiventrals in the tail region in the same manner as the neural arches (see above). Piiper (1928) and Wake and Lawson (1973) described the basiventrals as forming the parapophyses.

Like the theories of human vertebral development (see above), and contrary to statements made by several researchers (Williams, 1959; Jarvik, 1980; Verbout, 1985), there is little agreement between researchers as to the exact process of vertebral development in non-human tetrapods. There appears to be two main causes involved in the discrepancies between the theories: differences between the species and differences in the interpretation of vertebral development by the researchers. Variations in the terminology employed by the researchers appear to make the theories irreconcilable, however, close inspection of the theories indicates that the terminologies are compatible in most cases.

D. Phylogenetic Development of the Vertebral Centra

The phylogenetic development of the vertebrae, particularily the centra, has not been agreed upon by the researchers. Williams (1959: 30) stated that the confusion over the phylogeny of the vertebral column has been the result of a "lack of facts", particularly in regards to the development of the vertebral column in the fish. Likewise, Jarvik (1980: 153) stated that the "...knowledge of the vertebrae in the early tetrapods is still incomplete in many respects and we have to admit that we still cannot interpret safely the various types that have been described". Despite these problems, a general account of the development of the amniote centrum has been attempted. It is important to note that humans are not specifically referred to, but the discussions presented by Williams (1959) and Parke (1982) present the generalized condition in tetrapods and amniotes respectively.

According to Parke (1982) vertebral elements were first evident in Agnatha. Modern agnaths, the cyclostomes, possess two cartilaginous neural arches dorsal to the notochord and two hemal arches ventral to the notochord in each segment. Parke (1982: 14) stated that "...the neural arch has remained functionally and morphologically constant throughout the vertebrate lineage". It is therefore the centra which are the center of the controversy.

Parke (1982) stated that initially, the centra were composed of four components in fish: the dorsal and ventral

arch bases of the neural and hemal arches. The areas between these bases were filled in to form a definitive centrum. In the evolutionary development of the centra, the dorsal arch bases formed the pleurocentra, while the ventral arch bases formed the intercentra. The pleurocentra (and intercentra) of one segment either fused to form a single element or remained as two separate elements. Both the pleurocentra and intercentra might combine to form each centrum, or either the pleurocentra or intercentra disappeared and the centrum was formed mainly from one element or the other. In the reptiles the intercentra were thought to be the major component of the centra because they possessed hemal arches. This is in contrast to higher amniotes where the pleurocentra contributed to the majority of the centra. However, in the tail the intercentra were still present and possessed hemal arches. Parke (1982: 15) stated that "In most non-caudate mammals, the only discernible remnant of the intercentrum lies in the anterior arch of the atlas".

Williams' (1959) stated that only three elements were involved in the evolution of the tetrapod vertebrae: the neurapophysis, intercentrum and pleurocentrum. In the crossopterygians, which are the earliest known tetrapods, there were two centra per neural arch. The general evolutionary trend in the tetrapods, however, was one centrum per neural arch. In the early tetrapods there were two lines of development in the fossil record: one lineage died out while the other survived and later gave rise to the

mammals, birds and modern reptiles. It is interesting that in the former lineage the intercentrum formed the centrum whereas in the more successful lineage the pleurocentrum formed the centrum. Williams (1959) gave no explanation for the success of the pleurocentrum over the intercentrum in the early tetrapods.

It is unfortunate that neither Williams (1959) nor Parke (1982) discussed the human vertebral column in relation to the evolution of the column in the vertebrates. However, it can be assumed that human centra are composed of the pleurocentra.

III. MATERIALS AND METHODS

All of the embryos used in this study were from the Shaner collection of human embryos and fetuses, located in the Department of Anatomy at the University of Alberta. The 109 embryos which comprise this collection were acquired by researchers in the Department between 1915 and 1975. Notes on the embryos indicated that they were fixed in formalin (with the exception of a single embryo which was fixed in Bouin's), embedded in paraffin, serially sectioned for light microscopy and stained with hematoxylin and eosin. In some instances, photographs or scale drawings were made of the embryos prior to sectioning. There are some notes concerning the health and age of the mother prior to the abortion, but details of the abortions, which were apparently both spontaneous and induced, are absent in the majority of the cases. The CRL of the embryo, in addition to the plane and thickness of the sections, were recorded in all cases. Although there were no notes as to how the CRL of each embryo was measured, some of the drawings of the embryos indicated that this measurement was taken with the embryo in its natural position. That is, the embryos were not straightened before measuring the distance between the vertex and breech (Fig. 1).

Of the 75 embryos available between 2 and 23.5 mm CRL, 27 were chosen for this study. These embryos were chosen on the basis of:

1. the normalcy of the embryo: several of the embryos

were grossly deformed and could not be utilized. Only embryos which did not appear to have any obvious abnormalities were studied.

2. the general condition of the sections: many of the sections had sustained damage (such as tearing and folding) to the vertebral region, apparently during preparation and mounting. The embryos chosen had little (a small rip or tear in several sections) or no damage of these types in the vertebral region.

3. the amount of stain taken up by the sections: in some cases, all sections of an embryo were either very lightly or very darkly stained, which made it difficult to differentiate the tissues of interest. The stain had to be of such an intensity, neither very dark nor very light, that the vertebral elements could be distinguished with ease.

4. the obliquity of the sections: all of the embryos were sectioned obliquely to some extent, but in some cases the obliquity was so severe that appreciation of the structures was difficult. For this reason, only embryos in which the obliquity was at a minimum were chosen for observation.

Table 1 lists the embryos utilized in this study, with their CRL, plane and thickness of the sections. An important point concerns the plane of section and the curvature of the embryo. The term 'transverse' refers only to the body region of the embryos. Due to the curvature in the rump region this area was actually sectioned coronally, thereby producing a

complete transition from a transverse plane to a coronal plane in a single embryo. The neck region was often curved and tilted to one side in the embryos, producing sections which usually did not represent any standard plane (Fig. 2).

Before studying each embryo the slides were cleaned, but the mounting medium (Canada Balsa or Permount) had crystallized at the edges of the cover slip in many of the slides. Therefore, these sections could not be used as no attempt was made to clean the medium off the edges of the slides.

It was hoped that the entire vertebral column could be studied in the embryos. However, this was not always possible. Prior to chondrification, it was not possible to identify the first vertebra from the base of the occiput. Therefore in transversely-sectioned embryos with mesenchymal vertebral columns, the upper pharyngeal region was located and all sections caudal to this region were examined. In transversely-sectioned embryos in which chondrification had begun, the first vertebra was located and all sections caudal to this were studied. There were problems with the transversely and coronally sectioned embryos which impeded the study of regions of the vertebral column. Often in transversely-sectioned embryos, the neck was curved at such an angle that the sections were very oblique and, as a result, in most cases these sections were not utilized as understanding of the details was made extremely difficult. In coronally-sectioned embryos only the dorsal-most sections

with vertebral elements were studied as the curvature in the neck and rump regions produced sections which were very oblique and difficult to interpret. Occasionally an embryo was damaged in certain areas of the vertebral column, but if the remainder of the column had significant information, the embryo was included in the study.

Prior to examining the slides, specific questions were formulated based upon areas of controversy in the literature dealing with vertebral development. These questions involved the problem of whether or not resegmentation occurred during the blastemal stages of vertebral development and whether or not the intrasclerotomal fissures and intersclerotomal vessels and fissures existed in human embryos. These were specifically addressed during observation of each embryo, but the scope of the observations was not confined to these questions. Tissues surrounding the vertebral column (muscular, nervous and connective tissue) were studied and changes in them which appeared to affect the vertebral components were also recorded. As the sections of each embryo were studied with a Reichert binocular light microscope, a permanent record of the observations was produced on paper. Specific sections which illustrated particular observations were noted so that photographs could be taken of them at a later date. The photographs were taken using black and white Kodak Panatomic X film on a Leitz orthoplan photomicroscope.

The embryos were studied in series from the smallest to the largest CRL. In the majority of cases, it was found that the CRL was a good indicator of relative vertebral development: the larger the CRL, the more developed the vertebral elements. As information was gathered from the slides, developmental trends were rechecked in the previous embryos (for example, when the neural processes were first identified, the younger embryos were studied again for the development of this feature). As a result, all the embryos, in part or whole, were studied two or more times to ensure the accuracy of the interpretations.

The literature dealing with vertebral development which was reviewed prior to this research indicated that there were apparently no differences in the early blastemal development of the cervical, thoracic, lumbar, sacral and coccygeal vertebrae. Therefore, before the research was initiated, the assumption was made that all vertebrae develop in a similar manner during the blastemal stage. As a result, during this stage when specific vertebrae (for example, the fifth thoracic vertebra) could not be distinguished, the descriptions of vertebral development in each embryo were based on a composite of the entire column. When each region could be distinguished from the others (for example, when the ribs become prominent in the thoracic region) then the development of each region was described separately.

Although all the data presented in the results section was gathered from microscopic examination of the slides, in the initial stages of this study it was hoped to develop serial reconstructions of appropriate stages to aid in the understanding of the early development of the human vertebral column. The literature dealing with this subject outlines many problems and few reseachers, with the exception of Bardeen and Lewis (1901), Bardeen (1905a, b, 1908a), Wyburn (1944) and Sensenig (1949), used models as aids in the interpretation of vertebral development in serially sectioned human embryos. Bardeen and Lewis (1901), Bardeen (1905a, b) and Wyburn (1944) produced wax plate models and Sensenig (1949) constructed one transparent photographic model. Bardeen (1908a) did not describe the type of models he made. It was hoped that two and three-dimensional models would be of use in the understanding of this process, in addition to aiding the visualization of the relationships of the developing vertebral column and surrounding tissues. It is difficult to envision three-dimensional relationships from a series of two-dimensional sections and all three dimensions cannot, without difficulty, be conveyed to others. These problems, however, could be overcome with the use of serial reconstructions. Accordingly, solid model reconstruction in wax plates, graphical reconstructions on transparent paper, contour drawings and computer models were all used to produce two and three-dimensional representations of the

embryonic sections. (The specific methods used in these reconstructions are in the Appendix).

The first type of reconstruction attempted was a wax plate model of H104 using dental wax. The symmetrical nature of the vertebrae allowed the reconstruction of only the left-half of the first five thoracic vertebrae. The ribs, however, were not reconstructed due to the difficulties of fastening them onto the vertebral bodies with wax bridges and heated wires. Wax models have two major limitations. They are extremely fragile and respond to extremes in temperature by cracking in the cold and melting in the heat and, because the outlines of the structures were cut out in the wax, there were limitations as to what could be successfully and easily cut out. For example, small blood vessels and nerves were not easily cut out of the wax due to their size. Therefore, a different method of reconstruction was used which did not have these limitations.

Graphical models of H104, H42, H44 and H86 were reconstructed on transparency sheets. There were several advantages to this type of reconstruction over wax plate reconstruction. The projected sections were traced directly onto the sheets, whereas in wax plate modelling the sections were traced onto paper and then retraced onto the wax, increasing the number of steps and concomitantly the amount of error with each step. Furthermore, it was difficult to cut out small objects such as blood vessels and nerves in wax, but it is a relatively simple task to trace them onto

transparent sheets. The one disadvantage to graphical reconstructions was that they usually only represented one view of a structure. Solid model reconstructions were found to be more versatile in this respect as they could be manually rotated to any view, whereas this was not possible with graphical reconstructions.

Computer reconstruction was examined as an alternate technique as any view of the model could be produced on the screen. The first thoracic vertebra of H89 (44 mm) had been previously reconstructed by other researchers on a Hewlett Packard 9845B. There were several problems with the model, the main one being that the model did not have the illusion of being three-dimensional, but was flat with no depth to the image. This was unsatisfactory as the purpose of making models was to illustrate and clarify the stages of vertebral development. The computer models were confusing and did not help with these problems.

As on a graphics computer, a contour reconstruction can be produced by tracing the serial sections onto a single sheet of paper by hand, producing the same effect as a computer, but without the ability to rotate the image. This type of serial reconstruction was attempted on sections of H104, but it was found that it was not suitable for detailed reconstruction of the human vertebral column. Depth was not portrayed well in the contour drawing and the details became indistinguishable as more outlines were placed on top of each other.

It is the blastemal stage of vertebral development which has been the subject of the most controversy and it was hoped that models would provide a better understanding of this period. However, all of these methods of reconstruction were eventually abandoned because, although it was found that these methods were satisfactory when the vertebral elements were chondrified, they were of little use on embryos in which the vertebrae were composed of uncondensed, unchondrified sclerotome. In these embryos the boundaries of the mesenchyme were not well-defined and could not be accurately traced. To test the reliability of the tracings of uncondensed sclerotome, one section of H86 was retraced on five consecutive days, without referring to the previous tracings. On the fifth day, the five tracings were compared with each other and were found to be consistently different and it was concluded that uncondensed sclerotome was not conducive to accurate tracing with the methods available at that time. Therefore, methods of reconstruction which involved tracing unchondrified sclerotome were subsequently abandoned. Bardeen (1905a) also found this problem with the models he constructed from mesenchymal human vertebral columns.

One method of reconstruction which does not involve tracing sections is photographic reconstruction. This type of model was attempted from coronal sections of H58. With this method there were no problems related to tracing mesenchymal masses and this was its main advantage. However,

the disadvantage of this method was that the model was not transparent and it was therefore essentially the same as viewing the mounted sections themselves in sequence, except that the photographs were inferior in both contrast and detail. In an attempt to overcome this problem, two methods were tested: the first was to photocopy the photographs onto transparency sheets and the other was to trace the features of interest onto transparency sheets. Regardless of the photocopying machine used, the copies were not satisfactory as they did not retain the contrast found in the photographs and the areas of light stain, such as aggregations of loose sclerotome, did not reproduce well. The dark areas on the photograph, such as the dermatomyotomes, came out very dark on the photocopies and their accumulation over a series of photocopies prevented the viewing of details in that area on the photocopies below. The second method of tracing the sections from the photographs proved to be no more satisfactory than earlier attempts at tracing uncondensed sclerotome from projected sections. The unchondrified sclerotome in the photographs did not have the distinct boundaries necessary for accurate tracings.

The advantages of using models are several: they present a magnified two or three-dimensional representation of selected features of the sections and they are readily available to others in pictoral form, whereas the actual sections may not be available. Models, however, do not take the place of observation of the sections with a microscope,

but they aid in the understanding of the sections. As was previously discussed, the majority of the embryonic materials utilized in this study were young embryos with ill-defined mesenchymal vertebrae and therefore, reconstructions could not be utilized. As a result, the method of study involved observing the sections of each embryo with a light microscope. Black and white photographs were taken to illustrate the significant features of human vertebral development.

IV. RESULTS

Twenty-seven embryos ranging from 2 to 23.5 mm CRL were chosen for this study. The vertebral column and ribs were observed in sequence from the least to the most developed embryo. This sequence spans the development of the vertebrae from initial somite development to the onset of chondrification. The following is a description of the salient features observed in the vertebral columns and associated tissues of the embryos. The embryos are described in sequence from the least to most developed. In some cases, the specimens are sufficiently similar that a photograph of a single embryo has been used to demonstrate a particular feature present in several. Figure 39 diagrammatically illustrates the process of vertebral development as described in this section.

The somites, which give rise to the vertebral column and ribs in the human embryo, are first observed in H37 (2mm) (Fig. 3). This embryo is at the trilaminar disk stage where 3 layers, the ectoderm, mesoderm and endoderm, comprise the embryo. The somites are forming from the paraxial mesoderm which is located on either side of the notochord. The paraxial mesoderm is round in cross-section with a central, enclosed coelom and the cells of the somite are oriented radially around it. At the cranial and caudal ends of the embryo there is no indication of somites (Fig. 4).

Unlike H37, in H44 (3.5 mm), H86 (3.5 mm) and H32 (5.5 mm) the neural tube is closed in the cranial regions (Fig. 5), but remains open in the caudal aspect of these embryos (Fig. 6). The cranial somites of H44, H86 and H32 are open, parts of their ventral and medial borders having broken down (Fig. 5). The sclerotome cells from these somites are located between the remnants of the somite and the notochord. The notochord is separate from the ventral aspect of the neural tube, the dorsal aspect of the gut and the dorsal aortae and cardinal veins so the sclerotome cells have no opposition to aggregation around it. The notochord is already encircled by cells which are loosely aggregated (the medial sclerotome) when compared to those just ventral to the neural tube on the lateral edge of the embryo (the lateral sclerotome). It should be emphasized that the lateral and medial sclerotome masses are continuous and can be discerned most easily in transverse sections at this stage, although they are also distinguishable in coronal sections. As the cells condense around the notochord they leave behind them the dermatomyotome on the lateral side of the neural tube. Lateral to the neural tube the mesenchyme is more dense than that around the notochord. This is the region where the dorsal root ganglia develop from the neural crest cells. H44 has no dorsal root ganglia, but they are developing in H86 and H32.

Somites in the caudal or tail region are not open in H44, H86 or H32 (Fig. 6). These somites have a central

coelom and possess four borders in transverse sections: medial, lateral, ventral and dorsal. Somites in the tip of the tail are cuboidal or wedge-shaped in coronal and transverse sections, with the lateral and ventral borders combined as one.

Coronal sections (Fig. 7) of H44, H86 and H32 indicate the presence of aggregations of intersclerotomal blood cells, with no discernible vessel walls, extending from the lateral-most aspect of the sclerotome, opposite the dermatomyotomic junction, to the notochord. These blood cells, which appear to retain the original segmentation of the somites, form elongated groups of cells which extend to the aorta giving the appearance of being branches from it. The aggregations of blood cells extend throughout the majority of the ventrodorsal extent of the sclerotome cells. The mesenchymal cells between two consecutive aggregations of blood cells form sclerotomes. It should be emphasized that the medial and lateral sclerotomes both comprise each sclerotome.

The first clear indication of neural processes, the two dorsal extensions of the lateral sclerotomes, are observed in H42 (6mm) (Fig. 8). They are found throughout the length of the vertebral column in the transverse sections. Coronal sections of the caudal aspect of H42 (Fig. 9) show the presence of intersclerotomal blood cells extending the width of the sclerotome to the notochord. In addition, the sclerotomes are partially divided into cranial and caudal

halves of approximately equal extent by an incomplete intrasclerotomal fissure. The fissures are clefts which extend from the lateral-most aspect of the sclerotome, opposite the middle of the dermatomyotome, to approximately the medial edge of the lateral sclerotome. Just cranial to each fissure, on the lateral edge of the sclerotome, is the spinal nerve. All of the somites, except for those in the very tip of the tail, are open in this embryo.

Although the next embryo, H55, is 6 mm in CRL (as is H42), it is slightly more developed than H42. In coronal sections (Fig. 10), the intersclerotomal blood cells clearly have vessel walls and are now intersclerotomal arteries. They are branches from the aorta and appear to extend from the ventral aspect of the sclerotome throughout the majority of the width of the sclerotome. The intrasclerotomal fissures are also present and do not extend all the way to the notochord. The fissures are situated mainly in the middle of each segment and do not appear to reach either the ventral or dorsal borders of the sclerotome. A bulge in the medial aspect of the dermatomyotome lies opposite the fissure. Immediately caudal to the fissure, between it and the intersclerotomal artery, is a band of sclerotome cells which, under the light microscope, appear to be more dense and darker staining than the sclerotome band cranial to the fissure. These are the dark and light sclerotome bands respectively. In both bands the lateral aspects appear to be composed of more cells than medially. Therefore, the lateral

and medial sclerotome divisions are still discernible in both bands. Adjacent to the light band and cranial to the intrasclerotomic fissure lie the spinal nerves. The intersclerotomal arteries are opposite the junction of adjacent dermatomyotomes.

Costal processes, or ribs in the thoracic region, are initially observed in H66 (6.5 mm) (Fig. 11). They are ventral outgrowths of the lateral sclerotome, as are the neural processes which arise and extend dorsally around the neural tube and dorsal root ganglia. In this embryo the costal processes are found caudal to the level of the upper limb bud only and they can no longer be traced as the sections become coronal in the tail region. H28 and H93 (7 and 8.2 mm) also exhibit these ventral projections in sections caudal to the upper limb bud.

In the coronal sections of H66 (Fig. 12) the intrasclerotomal fissures extend all the way to the notochord. A myotomic bulge is found opposite the fissure and the spinal nerve is directly cranial to the fissure.

In H28 (7 mm) (Fig. 13) the dermatomyotomes have divided into two components and the myotomes have joined together as a long unbroken chain. There are notches in the medial aspect of the myotomic column at the region where the myotomes join together and myotomic bulges at the level of the intrasclerotomal fissures. Most importantly, in this embryo the dark and light bands of sclerotome have altered their position relative to the spinal nerves,

intrasclerotomal fissures and intersclerotomal arteries (Fig. 13): the artery is found within the substance of the light band while the fissure is situated in the middle of the dark band. The spinal nerve, which is directly cranial to the intrasclerotomal fissure, is now lying opposite both the dark and light bands; the upper part of the spinal nerve is opposite the caudal aspect of the light band while the lower part of the nerve is opposite the cranial aspect of the dark band. This is in contrast to earlier observations in younger embryos where the fissures are situated between the dark and light bands, with the arteries forming the cranial and caudal limits to each pair of bands and the spinal nerve is situated cranial to the fissure and opposite to the light band.

The costal processes, or ribs, are well-developed in H21 (8.5 mm) (Fig. 14) and are only found in the sections caudal to the brachial plexus. They are ventral extensions of the lateral sclerotome and there is no differentiation of the head of the rib from the sclerotome around the notochord. In coronal sections (Fig. 15) the sclerotome bands are identical to those found previously in H28. Part of the dark band immediately caudal to the fissure is found between the neural tube and dorsal root ganglia at the level of the intermyotomic gap (not shown in the figure).

The light and dark bands of sclerotome which, in younger embryos, could only be observed in coronal sections, have condensed sufficiently to be clearly visible in

transverse sections of H58 (9.4 mm). The neural and costal processes are situated at the level of the dark sclerotome band (Fig. 16). The light bands have no processes associated with them (Fig. 17). At this stage, the distinctions between the medial and lateral sclerotome divisions are lost.

In the coronal sections of H58 (Fig. 18) at the level of the notochord and dorsal to it, the caudal part of the dark band below the fissure extends between the myotomes and neural tube as the neural processes. They appear to be situated between adjacent myotomes, within the small, incomplete gap between them. There is no indication of ribs in the coronal sections. The distinction between the medial and lateral sclerotomes is no longer present in this embryo and those more advanced in development.

The head of the rib becomes more clearly defined in H34 (10 mm) and H50 (10.5 mm) as it initially begins to differentiate from the sclerotome of the vertebral body and intervertebral disk. Coronal sections of H34 (Fig. 19) also show the neural processes extending between the myotomes.

Transverse sections of H45 (11 mm) indicate that the light band of sclerotome is round whereas the dark band, with the neural processes and ribs in the thoracic region, is elongated laterally. A thin inconsistent band of cells from the notochord to the ventral and dorsal aspects of the light band is present in this embryo (Fig. 20).

The ribs appear to be placed at a higher level than the neural processes in H45, as the ribs are found in sections

cranial to those containing neural processes. Previously, both pairs of processes were observed to be at the same level. The spinal nerve follows the rib shaft from its origin at the neural tube (Fig. 21). The vertebrae cranial and caudal to the thoracic vertebrae do not have any indications of costal processes.

In the coronal sections of H45 (Fig. 22) the spinal nerve is found to be opposite the caudal-most aspect of the light sclerotome and the cranial-most aspect of the dark sclerotome. This situation is identical to that initially observed in of H28.

H43 (12.5 mm) exhibits the early stages of chondrification in the light bands of sclerotome. The heads of the ribs are not yet completely separate; a band of mesenchyme bridges the neural process and the head of the rib (Fig. 23). Studies of more advanced embryos indicate that this mesenchymal band is the anlage of the transverse process in the thoracic region. A blood vessel is found throughout the length of the vertebral column on the ventrolateral aspect of the sclerotomes. In the thoracic region, this vessel is found between the head of the rib and the rounded sclerotome.

The intersclerotomal arteries and intrasclerotomal fissures are no longer visible in the sections (Fig. 24). The caudal aspect of the light band is opposite the cranial half of the spinal nerve. The dark band is divided into two zones: a cranial zone A and a caudal zone B. The cranial

aspect of zone A is opposite the caudal half of the spinal nerve, but it also extends below the level of the spinal nerve.

The rib heads appear to be almost separate entities in H57 (14 mm). Again, there is a band of mesenchymal tissue bridging the gap between the head of the rib and the neural process.

The coronal sections of H57 (Fig. 25) are similar to those of H43. The caudal half of the light band is opposite the cranial half of the spinal nerve. Directly caudal to the light band is a very dark zone of unchondrified sclerotome cells (zone A) and directly below this area, and caudal to the spinal nerve, is a lighter zone of sclerotome cells (zone B) which is undergoing the initial stages of chondrification. Zone A is thinner in the notochordal region and widens laterally.

The sections of H19 (14.6 mm) (Fig. 26) reveal two distinct units: the vertebral body, which is undergoing chondrification, and the intervertebral disk, which is not. There are vessels on either side of the sclerotome in the middle of the vertebral bodies. The superior and inferior articulating facets are developing within a mesenchymal band which joins adjacent neural processes. The inferior articulating processes appear to be more developed than the superior ones (Fig. 27).

In the sagittal sections of H109 (12 mm) (Fig. 28) the suboccipital nerves, which are not associated with dorsal

root ganglia, are between the occiput and the first vertebra. The vertebral arteries are also present, arching over the seventh vertebra, lateral to the first six vertebral bodies. On either side of the vertebral body, opposite the center of the body, is a small artery. The first rib is opposite the seventh cervical vertebra (Fig. 29).

The vertebral bodies and intervertebral disks are well-developed in H60 (15 mm) and H88 (15 mm). In the coronal sections (Fig. 30) the lower part of the vertebral body is opposite the upper half of the spinal nerve and corresponds to the light band and zone B. The intervertebral disk, which corresponds to zone A, is opposite the caudal half of the spinal nerve and extends below the level of the spinal nerve. In H60, as in H57, zone A, or the intervertebral disk, is wider on the edges than in the middle. In H88, however, the disk is of approximately equal thickness throughout its width. The neural processes emerge from the cranial part of the vertebral body, just below the intervertebral disk.

Until H80 (17.5 mm) there is no clear evidence of transverse processes in any of the vertebra, other than the band of tissue between the rib and the neural process in the thoracic vertebrae. In this embryo (Fig. 31) the blood vessels found on the ventrolateral aspect of the cervical vertebral bodies are encompassed by transverse processes. Each transverse process extends from the neural process to

the lateral aspect of the vertebral body. There is no evidence of transverse processes in the lumbar vertebrae.

The transverse processes of the cervical vertebrae in H51 (15.5 mm) and H96 (20 mm) are cartilaginous. The blood vessels found in less developed embryos throughout the entire column are, in these two embryos, present only in the cervical region where they are located within the transverse foramina. The thoracic vertebrae of H51, H96 and H68 (20 mm) (Fig. 32) have cartilaginous transverse processes, emerging from the neural processes, which articulate with the ribs. The heads of the ribs articulate with the intervertebral disks and the adjacent vertebral bodies.

The articular processes of H17 (21.7 mm) are well developed and are undergoing chondrification within the bands of tissue between the neural processes.

The odontoid process, or dens, was not observed in any of the previously described embryos. In H81 (23.5 mm) the atlas vertebra does not possess a vertebral body (Fig. 33), although in younger embryos the first vertebra does have a vertebral body. The axis vertebra has an odontoid process with the notochord situated in the middle of it (Fig. 34). The lumbar vertebrae of this embryo do not yet possess chondrified transverse processes, but there is a ventrally directed mesenchymal projection from each of the lumbar neural processes. The sacral vertebrae of H81 (Fig. 35) are beginning to fuse together on their lateral-most aspects, but the vertebral bodies remain separated by intervertebral

disks.

H104 (22 mm) is slightly more advanced than H81. The vertebral arteries are encompassed by chondrified transverse processes (Fig. 36). The suboccipital nerves, which are not associated with dorsal root ganglia, are located between the base of the occiput and the atlas vertebra. The articulation of the rib with the vertebral bodies is similar to that found in the adult. Neurovascular bundles and muscle tissue are found in the intercostal spaces. (Fig. 37). In the lumbar region, the transverse processes are chondrified and muscle tissue is found between adjacent processes. Five sacral and several coccygeal vertebrae are present. The vertebral bodies and lateral aspects of the sacral vertebrae are fusing together (Fig. 38).

Summary

The Sclerotomes

In the early stages, the cells of the somites aggregate around the notochord and form a column encircling it. The presence of aggregations of blood cells indicate the original positions of the somites prior to their break-up. These blood cells and vessels segment the column of cells into sclerotomal units. There is no evidence of intersclerotomal fissures around the intersclerotomal vessels. Intrasclerotomal fissures, dark and light sclerotome bands and intersclerotomal arteries, which are branches from the aorta, occur shortly after the appearance

of intersclerotomal blood cells. Initially, the dark and light sclerotome bands are found within each segment. The light sclerotome is cranial to the intrasclerotomal fissure, as is the spinal nerve which is opposite the light band. The dark band is caudal to the fissure.

The bands of sclerotome undergo two changes from this point. At 7 mm the intersclerotomal arteries are found in the substance of the light band and the intrasclerotomal fissures in the dark band. The spinal nerve, which is directly cranial to the fissure, now lies opposite both the dark and light bands. By 12.5 mm the fissures and arteries are no longer present in the coronal sections. The spinal nerve is opposite the caudal-most aspect of the light band and the cranial-most aspect of the dark band. The dark band is divided into two zones: an uppermost dark zone, A, and a less dense zone, B, just caudal to it. Zone A is opposite to, and extends just below, the caudal aspect of the spinal nerve.

By 15 mm the intervertebral disks and vertebral bodies are well-developed. The disk corresponds to zone A and the vertebral body to both the light sclerotome band and zone B.

The Neural and Costal Processes

The neural and costal processes are projections from the lateral aspect of the sclerotomes. The neural processes first become visible at 6 mm. Coronal sections indicate that they are outgrowths of the dark sclerotome band which is situated caudal to the spinal nerve and intrasclerotomal

fissure. They extend dorsally to surround the neural tube. The transverse processes and the superior and inferior articulating processes are projections from the neural processes.

The ribs, or costal processes, are observed only in those sections caudal to the brachial plexus and they develop shortly after the neural processes first appear. Although initially the ribs appear to arise from the dark band of the sclerotomes at the same level as the neural processes, by 11 mm they appear to be situated at a higher level than the neural processes. By 22 mm, the ribs have assumed a position relative to the vertebral bodies and intervertebral disks similar to that found in the adult. There is no evidence of ribs or rib homologues in any region other than the thoracic.

V. DISCUSSION

As revealed in the literature review, the process of vertebral development in tetrapods is complicated and far from clear. Some of the differences between the theories in both human and non-human tetrapods can be attributed to varying interpretations of similar events, while others may be the result of the number of embryos studied. It is particularily important to have a closely graded series of embryos (Verbout, 1985) so that conjecture of the processes of development between available stages is either eliminated or at least reduced. Despite Williams' (1959), Jarvik's (1980) and Verbout's (1985) assertions that vertebral development is similar in all amniotes (and vertebrates in the case of Jarvik (1980)), the available literature points to the opposite conclusion.

Problems were encountered in this study when the results of other researchers were analyzed, as the majority of the pictorial material consisted of either drawings or small photographs. As a result, the observations of other researchers could not be satisfactorily investigated and confirmed with the photographs and diagrams provided. It was therefore impossible in many cases to determine why the differences in vertebral development had been reported and whether these were due to variations in interpretation or actual differences between species. However, wherever possible the original photographs and drawings were compared to those of the present investigation.

The following is a comparison of the results of the present investigation with those of other researchers studying both human and non-human tetrapods. The sclerotomes, vertebral bodies and intervertebral disks, and neural and costal processes are dealt with in turn. In addition, a discussion of the theory of resegmentation and a comparison of the present theory with known human congenital vertebral anomalies are presented.

A. The Sclerotomes

Many of the researchers studying vertebral development have assumed that the cells from the somite actively migrate to the notochord (Bardeen, 1905a; Piiper, 1930; Williams, 1942; Ehrenhaft, 1943; Sensenig, 1943, 1949, 1957; Peacock, 1951; Lawson, 1966). The current investigation was unable to determine the mechanism by which the sclerotome cells surrounded the notochord. However, it did appear that relative movement was occurring since the sclerotome cells were progressively closer to the notochord in subsequent embryos. However, this could be due either to the migration of the sclerotome cells or to the migration of the somitic derivatives away from the notochord, as Gasser (1979) proposed. Gasser (1979) studied serial sections of rat and human embryos in order to determine whether or not migration occurred in the sclerotome cells. By comparing magnified photographs of sections, he determined that the somites migrated dorsolaterally, depositing sclerotome cells behind

them, rather than the sclerotome cells migrating from the somites to the notochord. Gasser (1979) aligned the photographs using the notochord as the reference point, but did not explain the basis for assuming that the notochord was a static structure. Since the somites shift in position, it would seem reasonable to assume that the notochord may also migrate. Also, Gasser (1979) did not explain how the notochord and neural tube separated and sclerotome cells came to surround the notochord. Several points of reference, in addition to the notochord, such as the aorta and cardinal veins would ensure correct alignment of the sections in addition to indicating whether or not the notochord was a static structure.

The present investigation of human embryos does not support the presence of a perichordal tube, an independent structure from the sclerotomes, around the notochord as has been described in non-human embryos. Although in the early stages there was an area of lesser density surrounding the notochord - the medial sclerotome (Fig. 7), in more developed embryos this area was of equal density with the lateral sclerotome and there was no distinction between the two areas. Figures 24 and 25 demonstrate that the cells surrounding the notochord do not form densities independent of the lateral areas as has been reported in non-human embryos. For example, Verbout's figure 43 (1985: 48) demonstrates that the densities of the perichordal tube were at a higher level than the densities of the somitic

mesenchyme and that therefore they were independent structures. This phenomenon did not occur in the human embryos studied in the present investigation, but as demonstrated by figures 24 and 25, the dense areas extended from the lateral-most aspects of the sclerotomes to the notochord as continuous structures. None of the other researchers studying human embryos have reported the presence of an independent perichordal tube (Bardeen and Lewis, 1901; Bardeen, 1905a; Ehrenhaft, 1943; Wyburn, 1944; Sensenig, 1949, 1957; Peacock, 1951). However, all of the researchers studying non-human vertebral development did report its presence (Howes and Swinnerton, 1901; Piiper, 1928; Dawes, 1930; Mookerjee, 1930; Williams, 1942; Sensenig, 1943; Lawson, 1966; Werner, 1971; Wake and Lawson, 1973; Winchester and Bellairs, 1977; Dalgleish, 1985; Verbout, 1985). Of these researchers, only Howes and Swinnerton (1901), Werner (1971) and Winchester and Bellairs (1977) were not clear as as to whether the perichordal tube was completely sclerotomal in origin and only Mookerjee (1930) stated that the tube was partly sclerotomal and partly non-sclerotomal in origin. The reasons for the discrepancy between human and non-human embryos are not clear, but would seem to be attributable to differences between them. For example, Sensenig studied mice (1943) and humans (1949, 1957), yet only described the perichordal tube in mice. This tube appears to be the focus around which the vertebral centra and intervertebral disks develop in

non-human tetrapods. This focal center is apparently lost in humans and the entire sclerotome is the region from which the centra and disks develop.

The presence of intersclerotomal blood vessels and fissures has generally been reported in the literature. This study supports the presence of intersclerotomal vessels (which are branches from the aorta), but not intersclerotomal fissures. In the present investigation the intersclerotomal vessels were not encompassed by fissures. In some instances the vessels were devoid of blood cells, which were presumably removed during processing of the embryo, but vessel walls were clearly distinguishable (Fig. 22). As Verbout (1985) suggested, these empty vessels may have been mistaken for fissures by some researchers. It is interesting that the researchers studying amphibian vertebral development (Mookerjee, 1930; Lawson, 1966; Wake and Lawson, 1973) and one group studying reptile embryos (Howes and Swinnerton, 1901) did not mention the vessels and fissures. All other workers studying non-human tetrapods reported the presence of some, or all, of these features (see the review of the literature). Of all of the descriptions of human vertebral development, Sensenig (1949) was the only one to report the presence of both the intersclerotomal vessels and fissures, whereas all others (Bardeen and Lewis, 1901; Bardeen, 1905a; Ehrenhaft, 1943; Wyburn, 1944; Peacock, 1951; Sensenig, 1957) only discussed the intersclerotomal vessels. Sensenig's findings are

particularily interesting since he reported the intersclerotomal fissures in one human study (1949), but not in two others dealing with human (1957) and mice (1943) embryos. Verbout (1985) concluded that the intersclerotomal fissures were either artifacts, vessel lumina or optical illusions created by the arrangement of the nuclei. This would seem to account for the discrepancies in Sensenig's (1943, 1949, 1957) work and for the varying accounts of this feature in the literature dealing with non-human tetrapod development.

The present investigation concluded that the intersclerotomal vessels (Fig. 7) and intrasclerotomal fissures (Fig. 10) extended throughout the lateromedial and dorsoventral extent of the sclerotomes. As described in the review of the literature, few researchers studying human embryos were clear as to the precise extent of the vessels and fissures. Only Sensenig (1943, 1949, 1957) clearly stated that the intersclerotomal vessels formed incomplete boundaries between the sclerotomes. This conclusion is not in agreement with the current findings, although the reasons for this discrepancy are not clear. Dalgleish (1985) stated that the vessels did not prevent the intermingling of cells between adjacent segments, but did not provide any evidence to support this statement. It would seem to be difficult to determine this from static sections.

The intrasclerotomal fissures are also features of considerable controversy. This study supports their presence

in human embryos and indicates that they are not artifactual as both Dalgleish (1985) and Verbout (1985) have proposed. The fissures were consistent features in the sclerotomes in frontal sections of the embryos studied, appeared prior to the definition of the dense bands (Fig. 9) and were still present after they were encompassed by the dark band (Fig. 15). This is contrary to Dalgleish's (1985) and Verbout's (1985) statements that the fissures were artifacts produced by the varying densities in each sclerotome. According to their proposal, the fissures would not be visible either prior to the appearance of the dark and light bands or after the dark bands had surrounded the fissures. Yet in the present investigation, the fissures were clearly present in both instances.

The present research has concluded that the sclerotomes were not of equal density throughout their blastemal stages, but develop light and dark bands (Figs. 10, 13, 24, 25). A similar phenomenon has been reported by all other workers studying both human and non-human vertebral development, with the exceptions of Howes and Swinnerton (1901), Mookerjee (1930), Lawson (1966) and Wake and Lawson (1973), who studied either amphibians or reptiles. The reason for this discrepancy is not clear, although it may be a result of the species studied. The reasons for the appearance of light and dark bands could not be determined as the thickness of the sections did not permit observation of the nuclei for picnotic or mitotic changes. Flint (1977) studied

this phenomenon in mouse embryos and came to the conclusion that the densities were the result of the growth of the spinal ganglion and nerve through the cranial half of each sclerotome, which displaced the cells laterally. However, if the cells were pushed to the side as the nervous tissue grows through the cranial sclerotome half, the cranial half should become more dense rather than the caudal half. Flint (1977: 477) did not explain how "...forcing cells to either side..." of the spinal nerve produced a dense caudal sclerotome half.

There is also some controversy over the position of the dark and light bands. This study indicates that the dark bands were initially confined to the caudal half of each sclerotome below the intrasclerotomal fissure, while the light bands were confined to the cranial half of each sclerotome above the intrasclerotomal fissure (Fig. 10). However, these bands were observed to change positions relative to the arteries and fissures, with the light bands coming to surround the intersclerotomal arteries and the dark bands encompassing the intrasclerotomal fissures (Figs. 13 and 15). The position of the spinal nerve in each cranial sclerotome half was carefully noted prior to, and following, the relative position changes of the bands. The location of the spinal nerve was used as further evidence that changes had occurred. The consistency of the spinal nerves was based on the work of Keynes and Stern (1984) who studied them in chick embryos. They found that the spinal nerve consistently

passed through the cranial sclerotome half and in only six of seventy-three somites were any of the sensory nerve fibers found in the caudal sclerotome half. The motor nerve fibers did not deviate from the cranial sclerotome half. Several others also stated that the spinal nerve occupied the cranial half of the segment (Bardeen, 1905a; Piiper, 1928; Dawes, 1930; Williams, 1942; Sensenig, 1943, 1949, 1957; Flint, 1977; Verbout, 1985). Based on the results of Keynes and Stern's (1984) work and the observations of this study, the spinal nerves were thought to be of consistent placement and were therefore used a landmarks in the embryos. To ensure that the obliquity of the spinal nerves was at a minimum, only those sections in which the notochord was coronally sectioned were analyzed. Initially, the spinal nerve was located cranial to the intrasclerotomal fissure and opposite the light band in the cranial sclerotome half. (Fig. 10). However, in older embryos in which a relative change in the positions of the bands had occurred, the spinal nerve was opposite the caudal aspect of the light sclerotome band and the cranial aspect of the dark band, while retaining its position cranial to the intrasclerotomal fissure (Figs. 13 and 15).

This phenomenon of the changing positions of the densities appears to be exclusive to human embryos as it has not been reported by anyone studying non-human vertebral development. Bardeen (1905a) and Ehrenhaft (1943) described a loss of cells from the caudal sclerotome half. Bardeen

(1905a) reported that the caudal densities were then reinforced by tissue from the cranial sclerotome halves, while Ehrenhaft (1943) stated that the dense zone shifted cranially. It was not described by either researcher how cellular movement could be determined from static sections. This is a similar phenomenon to that described in the present investigation (although actual movement of cells could not be determined in the sections): the intrasclerotomal fissures were encompassed by the dark band. Wyburn (1944), Sensenig (1949) and Peacock (1951) stated that the dense bands were a combination of the cranial and caudal sclerotome halves of the same segment and although they did not report any shifting, the result was the same – the intrasclerotomal fissures were surrounded by the dark sclerotome bands.

Verbout (1985) rejected all reports of changes in the position of the densities, stating that this was a result of the misinterpretation of two separate densities in the perichordal tube and lateral areas as a single density. However, there is no evidence for a perichordal tube in human embryos. In addition, Verbout's figures 15 (1985: 22) and 27 (1985: 35) indicate that there has been a change in the positions of the bands similar to that described here, which he has apparently not included in his interpretation.

Bardeen and Lewis (1901) and Sensenig (1957) were the only researchers not to describe the dense areas as being intrasegmental in position. Bardeen and Lewis (1901)

reported that only the caudal third of each sclerotome was dense, while Sensenig (1957) stated that the caudal sclerotome half was dense. These differing reports would seem to be the result of varying interpretations, particularly in the case of Sensenig who studied human embryos on two occasions (1949, 1957) and came to different conclusions in both studies.

It is interesting to note that non-human studies (see literature review) did not report any alterations of the density patterns. The most plausible explanation for this discrepancy between human and non-human embryos would seem to be that human vertebral development is different from that of other tetrapods.

The current investigation has revealed that the dense sclerotome bands were subdivided into two zones: a dense cranial zone A (the intervertebral disk) and a less dense caudal zone B (part of the centrum) (Figs. 24 and 25). No others have reported a similar phenomenon in any tetrapod, including humans. However, Wyburn (1944) and Sensenig (1949) described three zones (cranial, middle and caudal) in each dense band, the middle of which remained as the intervertebral disk and those cranial and caudal to it contributing to the vertebral bodies in human embryos. The photographs provided by Wyburn (1944) and Sensenig (1949) are small and unclear, but suggest that the cranial and caudal zones may be areas of the chondrifying vertebral bodies which are in a less advanced state of chondrification

than the central region, thereby giving the impression that they are part of the dense bands. The zones described in the present research appeared, and were very clear, prior to chondrification of the dark bands. It is interesting that several researchers (Piiper, 1928: Dawes, 1930; Williams, 1942; Sensenig, 1943) also observed three zones in the perichordal rings of the perichordal tube, but they were not evident in the photographs of Dawes (1930), Williams (1942) and Sensenig (1943) nor in the diagrams provided by Piiper (1928). It was therefore impossible to compare these three zones to those reported by Wyburn (1944) and Sensenig (1949) and to the two zones observed in the present investigation.

B. The Vertebral Bodies and Intervertebral Disks

This study indicates that the vertebral centrum was derived from the light sclerotome band and zone B of the dark band cranial to it. Zone A of the dark band remained unchondrified and formed the intervertebral disk (Fig. 39). The intersclerotomal arteries and intrasclerotomal fissures had disappeared by the stage when the two zones were distinguished in the dark band. However, since zone A extended a short distance below the level of the spinal nerve it would appear that zone A was not strictly confined to the area of the dark band cranial to the fissure, but extended into the area of the fissure and slightly below it (compare the level of the fissure in Fig. 18 to that of the level of zone A in Figs. 24 and 25). Therefore, if the

intrasclerotomal fissures were still present in the sections, zone A would surround the fissure (Fig. 39).

No other workers studying human vertebral development have come to the same conclusion regarding the formation of the vertebral centra and disks. However, these results appear to concur with those of Bardeen (1905a), Ehrenhaft (1943) and Peacock (1951) who all concluded that the intervertebral disks were derived from the dense tissue around the intrasclerotomal fissure while the remainder of the sclerotomes formed the vertebral bodies. However, none of these investigators described the differing zones in the dark bands. It is possible that this discrepancy is due to these investigators not distinguishing the two zones in the dense band. Wyburn (1944) and Sensenig (1949) agreed that the central portion of the dark band formed the intervertebral disks, whereas the present investigation indicated that it was the cranial aspect of the dense zone which formed the disks. In Wyburn's (1944) and Sensenig's (1949) investigations, the cranial and caudal aspects of the dark zones both contributed to the vertebral bodies. In the present study, however, only the caudal aspect of each dark band contributed to the vertebral bodies. It is interesting that in 1957 Sensenig came to a different conclusion than in 1949 (see above); in the former study the dense caudal sclerotome half formed the intervertebral disk, as well as the cranial and caudal aspects of the adjacent vertebral bodies. The light areas also contributed to the vertebral

bodies. The discrepancies between his two theories appear to be the result of differing interpretations. Bardeen and Lewis (1901) stated that part of the dense caudal third of each segment formed the intervertebral disks while the remainder of the disk and light areas formed the neural processes and vertebral bodies. In the present investigation the caudal third of each segment was not dense, but was part of the light band (Fig. 15).

Comparison of the present results with those of researchers working with non-human embryos was not possible due to fundamental differences, such as the presence of the perichordal tube, between the two groups. A general similarity is that the perichordal disks described by most researchers (see the review of the literature) were intrasegmental and formed the intervertebral disks, while the A zones of the dark sclerotome band in the present embryos were also intrasegmental and formed the intervertebral disks. In addition, the vertebral bodies were intersegmental structures in the embryos under current investigation and in the majority of the non-human tetrapod embryos.

C. The Neural and Costal Processes

The present study supports the work of others in non-human (Sensenig, 1943; Werner, 1971; Dalgleish, 1985; Verbout, 1985) and human embryos (Bardeen and Lewis, 1901; Bardeen, 1905a; Ehrenhaft, 1943; Wyburn, 1944) that the

neural processes were derivatives only of the caudal sclerotome halves (Fig. 19). These findings do not agree with those of Piiper (1928), Mookerjee (1930), Williams (1942) and Winchester and Bellairs (1977) in non-human tetrapods and Sensenig (1949, 1957) in humans where it was stated that the neural processes were intersegmental structures, arising from the caudal sclerotome half of one segment and the cranial sclerotome half of the next segment. In the present study, the neural processes were only projections from zone B of the dark bands and, if they were intersegmental, as has been suggested, they should then be formed from the light bands which surround the intersclerotomal arteries. Nor do these results concur with those of Dawes (1930) who considered the neural processes to be intrasegmental, since they did not extend cranial to the intrasclerotomal fissure. Howes and Swinnerton (1901), Peacock (1951), Lawson (1966) and Wake and Lawson (1973) were not sufficiently clear as to the exact origin of the processes for comparison with the present work.

The costal processes were outgrowths of the caudal sclerotome halves in the embryos of the present investigation. This finding is in agreement with Piiper (1928), Dawes (1930), Williams (1942), Werner (1971) and Verbout (1985) in non-human tetrapods and Bardeen and Lewis (1901), Bardeen (1905a), Ehrenhaft (1943) and Wyburn (1944) in humans. Sensenig's work on mice (1943) and human embryos (1949) indicated that the costal processes were

intersegmental structures. However, in the present study the costal processes were initially outgrowths of zone B of the dark sclerotome band and therefore could not be intersegmental structures. Howes and Swinnerton (1901), Mookerjee (1930), Peacock (1951), Lawson (1966), Wake and Lawson (1973), Winchester and Bellairs (1977) and Dalgleish (1985) did not describe the origins and early development of the ribs in sufficient detail for comparison.

It is interesting that some human anatomy texts (for example, Grant, 1951; Anderson, 1983) state that the transverse processes of the cervical, lumbar and sacral vertebrae are composed of the rib rudiments fused to the transverse elements – two elements that are separate entities in the thoracic region. In the present investigation there was no evidence of costal processes in any region other than the thoracic. Bardeen (1905a, 1908a, b) was the only researcher to describe this dual composition in human embryos. Others (Wyburn, 1944; Sensenig, 1957) also described costal processes in the cervical region of human embryos, but did not describe their fate. No photographs of costal processes in regions other than the thoracic were provided by researchers who described their presence, so comparisons between these and sections from the present study were impossible.

Unlike humans, non-human tetrapods often have ribs throughout the entire vertebral column (Wake, 1979) and Dawes (1930) and Verbout (1985) described the cervical

costal processes as forming part of the arterial canal with the transverse processes. Verbout (1985) stated that the costal processes of the cervical and lumbar regions fused to the neural processes. The present investigation does not support the presence of ribs or rib homologues in any region of the human vertebral column other than the thoracic.

As described in the literature review, the origins of the articular processes are extremely varied. The present study could not determine the origin of the mesenchymal band between the neural processes from which the articular processes arose.

D. A Review of the Theory of Resegmentation

As is evident in the review of the literature, the majority of the tetrapod vertebral development theories indicate that the centrum ultimately forms from two adjacent segments. Verbout (1976) credits Remak (1855) with the original concept of resegmentation in the vertebral column. Remak (1855, cited in Verbout, 1976) used the theory of resegmentation to bring the neural arches at the caudal part of each somitic segment onto the cranial aspect of each vertebral body. His (1868, cited in Verbout, 1976) also referred to resegmentation, but used it to explain the alternation of muscles and vertebrae necessary for movement of the bones. Sensenig (1943) agreed with this latter argument, stating that since the original segments were intrasegmental in relation to the myotomes, resegmentation

was necessary to bring the vertebrae into an intersegmental postion for reasons of muscular action. Von Ebner (1888), according to Verbout (1976), was the first to describe the presence of the intrasclerotomal, or intervertebral, fissures, which were used as evidence for the process of resegmentation.

The only workers to disagree with the theory of resegmentation in their theories of vertebral development were Wake and Lawson (1973), Dalgleish (1985) and Verbout (1985) who all studied non-human tetrapods. Wake and Lawson (1973) stated that since the sclerotome cells were not initially segmented they were unable to demonstrate any evidence of resegmentation. This also seems to be the case of Howes and Swinnerton (1901) who only described their segments as comprising an intercentrum, a centrum and neural arch. However, they neither denied nor confirmed that resegmentation had taken place. Verbout (1976, 1985) also used the same argument as Wake and Lawson (1973) against resegmentation in regards to the perichordal tube of sheep embryos. Earlier, however, Verbout (1976:220) concluded that the concept of resegmentation was "...in all probability invalid". He also studied several other amniote embryos, including humans, and came to the conclusion that vertebral development was the same in all amniotes, thereby extending his argument against resegmentation to humans and other amniotes (Verbout, 1985).

Dalgleish's (1985) argument against resegmentation was based on evidence gained from autoradiographic studies. He stated that if the vertebral centra were intersegmental structures, the centers of chondrification would be placed between the dense perichordal disk and the loose perichordal disk. However, as revealed by autoradiography, the centers were initially located only in the loose perichordal disks. Dalgleish (1985) seemed to be referring to the dense and loose perichordal disks themselves as segments and failed to take into account that, as discussed in the review of the literature, other researchers based the intersegmental nature of the centra on the observation that the future vertebral body regions of the perichordal tube were derivatives of two sclerotomal segments. That is, the vertebral regions (and perichordal disks) were not themselves segments, but were parts of the sclerotomal segments. Furthermore, since Dalgleish (1985) described the dense perichordal disks (the intervertebral disks) as being adjacent to the junction of the cranial and caudal halves of the sclerotomes, these were intrasclerotomal structures and the loose perichordal disks (the future centra) intersclerotomal structures. It is generally accepted that the centers of chondrification occur in the middle of the vertebral bodies and not at the edges, so it would not be expected that the centers would occur at the junction of the loose and dense disks as Dalgleish (1985) stated, since the dense disks did not form the centra. If Dalgleish (1985)

were to state, as did Wake and Lawson (1973) and Verbout (1976, 1985), that the basis for the rejection of resegmentation was that there was initially no segmentation in the perichordal region, and therefore no resegmentation, his argument would be more reasonable. Dalgleish (1985) also stated that since the neural arches were already in a position between two myotomes without resegmentation that this process need not occur. However, he failed to note that it is most often the vertebral bodies which are involved in resegmentation, not the neural processes (see the review of the literature).

The autotomous tail vertebrae present in some lizards have been cited as evidence for the process of resegmentation. Howes and Swinnerton (1901), Werner (1971) and Winchester and Bellairs (1977) all reported the presence of autotomous tail vertebrae in their specimens. Both Werner (1971) and Winchester and Bellairs (1977) stated that the intersclerotomal fissures in these vertebrae were not obliterated in development as they were in other areas of the vertebral column, but remained as part of the autotomy split in the middle of the centrum. Verbout's (1985) argument against this was that there was no segmentation by means of fissures and vessels in the perichordal tube in the sheep embryos which he studied. However, this does not necessarily mean that these features did not occur in reptiles. The arguments for and against the importance of the autotomy split in reptiles and for resegmentation in

general appear to be the result of differing interpretations between the researchers.

The problem which needs to be, and has not yet been addressed, is how to prove that the sclerotome cells from each somite retain the original somitic segmentation, or how to prove that they do not. Until this has been attended to, the theories of vertebral development will be split by those who believe in resegmentation and those who do not. All the researchers who described the vertebral body as intersegmental, including this present study, have done so on the tacit assumption that the sclerotome cells remain in the segment opposite the somite from which they originated. In the present research, this assumption was based on the position and morphology of the intersclerotomal vessels. These were present between the somites, or their derivatives, in all of the embryos up to the onset of chondrification, with the exception of the very earliest one in which the somites were just developing. It would seem reasonable that the vessels provide boundaries which prevent extensive exchange of sclerotome cells between adjacent segments. A small amount of intermingling between segments does not seriously affect the theory of resegmentation, but if the sclerotome cells from, for example, the tenth somite intermingle and are located opposite the fifth somite, or the thirteenth, then the theory of resegmentation would no longer be valid. The absence of vessels or fissures in the axial region does not prove that the cells in this area are

not from the somite opposite them. Until this can be proven, the theory of resegmentation, or the recombination of two adjacent segments to form one centra, would seem reasonable based on the evidence of the present investigation.

E. A Comparison of the Congenital Vertebral Anomalies of Formation with the Theory of Vertebral Development

None of the researchers have compared their theories of vertebral development with the known human congenital vertebral anomalies of formation to test whether their theories can account for these anomalies. An attempt to reconcile the present theory with these anomalies is presented. Unfortunately, little is known of their etiologies at the present time.

Tsou et al. (1980) have documented many congenital vertebral anomalies found to occur in humans and have divided these anomalies as to whether they arise in the embryonic (up to 56 days gestation) or fetal periods (57 days gestation until birth) of development. The former anomalies of formation are mainly those of hemivertebrae and hemimetamer aplasia, or ipsilateral aplasia of the centrum and neural arch, and hemimetamer hypoplasia, or the partial diminution of one lateral half of the centrum and neural arch. In the latter period the defects include partial absence of the centrum with a complete neural arch. These fetal-period anomalies are thought to be the result of atypical chondrification and ossification (Tsou et al.,

1980) and will not be dealt with here.

Tsou et al. (1980) have proposed two theories to explain the cause of single hemivertebrae and hemimetamer aplasia (these appear to be the same anomaly, but apparently have been differentiated because they have different etiologies). One theory is that due to retarded development in one somite of a pair, the less advanced somite shifts caudally, leaving the other somite without a partner to pair with, resulting in the development of a single hemivertebra. The other theory is that of somite aplasia which leads to hemimetamer aplasia of the centrum. Ehrenhaft (1943: 290) also proposed that wedge vertebrae occurred as a result of the shifting of "the vertebral segments" cranially which "...will cause an anlage for a half vertebrae to remain at the lower and the upper end of the unequally shifted column...". Therefore, in this theory one entire side of somites shifts cranially, leaving one somite at the cranial-most and another at the caudal-most aspects of the column, both of which form hemivertebrae. In contrast, Tsou et al. (1980) proposed that only some of the segments shift caudally. Their diagram (Fig. 4, Tsou et al., 1980: 218) has ten somites on the "normal" side and only nine segments on the shifted side and it was not explained why one somite was absent. This diagram shows the first five somites to be perfectly paired, the next three to be offset in an intersomitic position in relation to the normal somites and the last (ninth on the shifted side) to be perfectly matched

with the tenth somite of the normal side. It would seem unusual that only some of the somites would shift (the sixth, seventh and eighth ones) and that the somites caudal to these shifted ones (the ninth somite in their diagram) would remain in normal alignment with their partners. It was not explained how some of the somites (the sixth, seventh and eighth on the shifted side), which were displaced by one-half of a segment to those on the normal side, could develop in a typical manner considering that they were not in normal alignment with the somites opposite them. This theory seems to be unlikely when compared to that of Ehrenhaft (1943).

Tsou et al. (1980) were not specific as to the cause of hemimetamer hypoplasia, but suggested that it was due to problems with the sclerotome cells. They also did not explain the etiology of multiple hemivertebrae.

The present investigation suggests that unilateral absence of one somite of a pair would result in two deformed vertebrae and a deformed intervertebral disk between them. The cranial-most vertebra would be normal in its upper aspect since it is derived from a different segment, but deformed caudally: one lateral half of its caudal aspect would be missing. The intervertebral disk would consist only of one lateral half and the vertebra caudal to the disk should be missing one-half of its cranial aspect, including part of the neural arch. However, the caudal aspect of this vertebrae would be normal since it is formed from a

different segment. Therefore, unlike Tsou's et al. (1980) and Ehrenhaft's (1943) theories, two abnormal vertebrae, although not hemivertebrae, would be the result of the absence of one somite.

Hypoplasia of one somite of a pair would also result in vertebral deformities, although not hemivertebrae. If the number of sclerotome cells is diminished, but the hypoplastic somite still contributed equally to both the cranial and caudal sclerotome halves, the same type of deformities should result as above, but the severity should be decreased. If the hypoplastic somite were to contribute sclerotome cells only to the cranial half of the sclerotome, leaving the caudal half devoid of cells, the intervertebral disk might be slightly diminished in its cranio-caudal extent. The vertebra cranial to the disk should be normal. However, the vertebra caudal to the disk would be deficient in its cranial aspect: one-half of the neural arch and vertebral body would be missing. The caudal aspect of the vertebra should be normal as it is formed from the next caudal segment. If the hypoplastic somite were to contribute cells only to the caudal sclerotome half, then one lateral half of the intervertebral disk would be absent, as would one half of the caudal aspect of the vertebral body cranial to the disk. The cranial half of this vertebra should be normal as it is formed from a different segment. The vertebral body below the disk would also be normal as it is formed from the caudal half of the segment and the cranial

half of the next normal segment.

According to the currently proposed theory of vertebral development, ipsilateral absence of one vertebra could only occur if two adjacent somites on one side of the embryo were hypoplastic and the cranial somite contributed cells only to the cranial half of its segment and the caudal somite only contributed cells to its caudal sclerotome half. This would result in the ipsilateral absence of the sclerotome cells of two adjacent sclerotome halves from different segments which normally contribute to one vertebral body and the intervertebral disk below it.

Unfortunately, it is difficult to reconcile the predicted anomalies, as described above, with those which are actually found to occur in the human vertebral column. However, it is interesting to note that Nasca et al. (1975) have suggested that some hemivertebrae (supernumerary hemivertebrae) are the result of the splitting of the centers of chondrification while others (wedge-shaped hemivertebrae) are attributed to the failure of one of the centers of chondrification to appear. It may therefore be that hemivertebrae are not the result of somitic or sclerotomic deficiencies. Although the present theory can account for these congenital anomalies of formation, more research into the etiologies of these defects is needed in order to determine whether the primary cause of these anomalies is somitic, cartilaginous or osseous, before they can be used to assess the accuracy of any theory of

vertebral development.

VI. SUMMARY

The vertebral columns of twenty-seven serially sectioned human embryos ranging from 2 to 23.5 mm CRL were studied with a light microscope. Reconstructions of the early stages of vertebral development were attempted, but were unsuccessful due to the uncondensed nature of the tissue. Therefore, documentation of the process of vertebral development was accomplished through detailed notes and photographs. Both the blastemal and cartilaginous stages of vertebral development were investigated and particular attention was paid to the vertebral bodies, intervertebral disks and the neural and costal processes throughout the vertebral column. The main purpose for undertaking this research was to determine if the process of resegmentation, or recombination of the sclerotome segments, occurred in the blastemal stages of human vertebral development. Other problems investigated focused on determining the presence of intersclerotomal vessels and fissures and intrasclerotomal fissures.

The cranio-caudal gradient of development in each embryo was an asset as several stages of development were represented in a single embryo. Likewise, the curvature of the embryos was also an asset as it resulted in two planes of sectioning, transverse and frontal, in many of the embryos. This allowed observation of the process of vertebral development in several planes, enhancing the understanding of the sections.

The discussion focused on comparing the theories put forth by others in both human and non-human tetrapods with the results of the present investigation. The most striking observation with comparison to the literature was that it appears, contrary to Williams' (1959), Jarvik's (1980) and Verbout's (1985) conclusions, that human vertebral development is not identical to that of other vertebrates or amniotes. The most obvious difference between human and non-human tetrapod vertebral development is the lack of a perichordal tube in humans. In other tetrapods, this tube is the central axis of centrum and intervertebral disk development, whereas in humans the entire sclerotome is involved in vertebral formation.

Congenital vertebral anomalies were briefly compared to the present theory. It was found that there is not enough known about the etiology of hemivertebrae and hemimetamer hypoplasia and aplasia to either confirm or deny the accuracy of the present theory. And, there are indications that these anomalies may be the result of abnormal chondrification, rather than atypical development of the somites and sclerotomes.

The conclusions drawn from this investigation were that the intersclerotomal arteries and intrasclerotomal fissures do exist in human embryos, but the intersclerotomal fissures do not and that resegmentation of the sclerotomes does occur during the blastemal stage of human vertebral development. If the primary segmentation of the sclerotomes is defined by

the intersclerotomal arteries and somites, the definitive vertebral bodies do not correspond to this segmentation, but rather to the caudal half of the segment above and the cranial half of the segment below.

VII. TABLES

TABLE 1

The CRL, Plane of Section and Section Thickness of the Embryos

EMBRYO NUMBER	CRL (mm)	PLANE OF SECTION	SECTION THICKNESS(μm)
H17	21.7	coronal	20
H19	14.6	coronal	25
H21	8.5	transverse	12
H28	7.0	transverse	10
H32	5.5	transverse	10
H34	10.0	transverse	10
H37	2.0	transverse	10
H42	6.0	transverse	10
H43	12.5	transverse	10
H44	3.5	transverse	10
H45	11.0	transverse	10
H50	10.5	transverse	10
H51	15.5	transverse	20
H55	6.0	transverse	10
H57	14.0	transverse	12
H58	9.4	transverse	12
H60	15.0	transverse	12
H66	6.5	transverse	10
H68	20.0	coronal	12
H80	17.5	transverse	15
H81	23.5	transverse	20
H86	3.5	transverse	10
H88	15.0	transverse	15
H93	8.2	transverse	10
H96	20.0	transverse	20
H104	22.0	sagittal	10
H109	12.0	sagittal	10

VIII. FIGURES

The abbreviations used in these figures are located after the List of Figures.

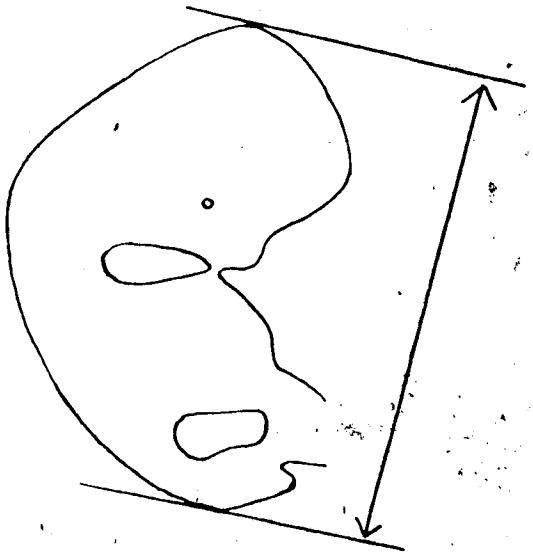
With most of these figures is a tracing of a diagram made of each embryo prior to sectioning. Each tracing is of the embryo in lateral view with the approximate level and angle of the section indicated by a line. Drawings of the embryos are not provided for photographs of sagittal sections.

On the coronal sections a star indicates the cranial aspect of the section.

The magnification bars on each photograph represent one millimeter.

Figure 1
A copy of a sketch of H80 (17.5 mm) prior to sectioning indicating the method of measuring the CRL of the embryo. The CRL, the distance between the vertex and breech, has been measured without straightening the curled embryo.

Figure 2
A copy of a sketch of H80 (17.5 mm) showing the several planes of sectioning which result from the curvature of the embryo. Due to the curvature of most embryos at the neck and rump, several planes are produced when the trunk of the embryo is sectioned transversely.
A. The sections in the head region are very oblique and do not represent any particular standard plane.
B. The sections in the body are transverse.
C. The sections at the rump are coronal.



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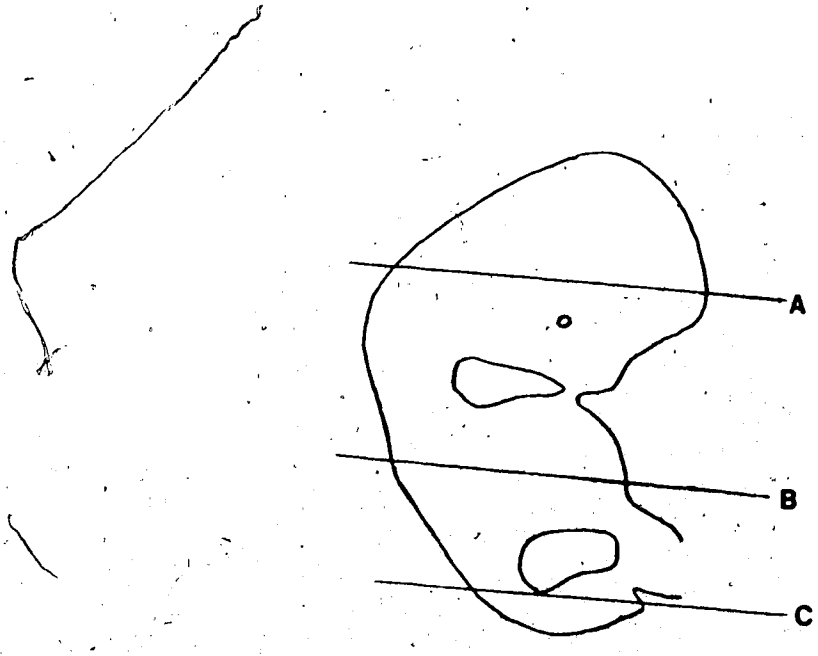

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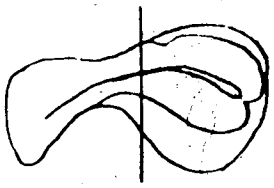
Figure 3
H37 2 mm Transverse Section
This embryo is at the trilaminar disk stage with three layers of cells present: the ectoderm, endoderm and mesoderm. The neural tube is forming from the ectoderm. The somites are derived from the paraxial mesoderm which is on either side of the notochord (which has been removed presumably during processing of the embryo). There is a small central coelom in the center of the somite on the right.

Figure 4
H37 2 mm Transverse Section
This section is from an area of the same embryo shown in figure 3 in which somites have not formed from the paraxial mesoderm. Note that the three layers of the trilaminar disk are well developed.

3



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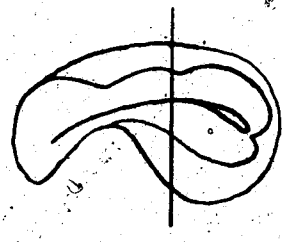
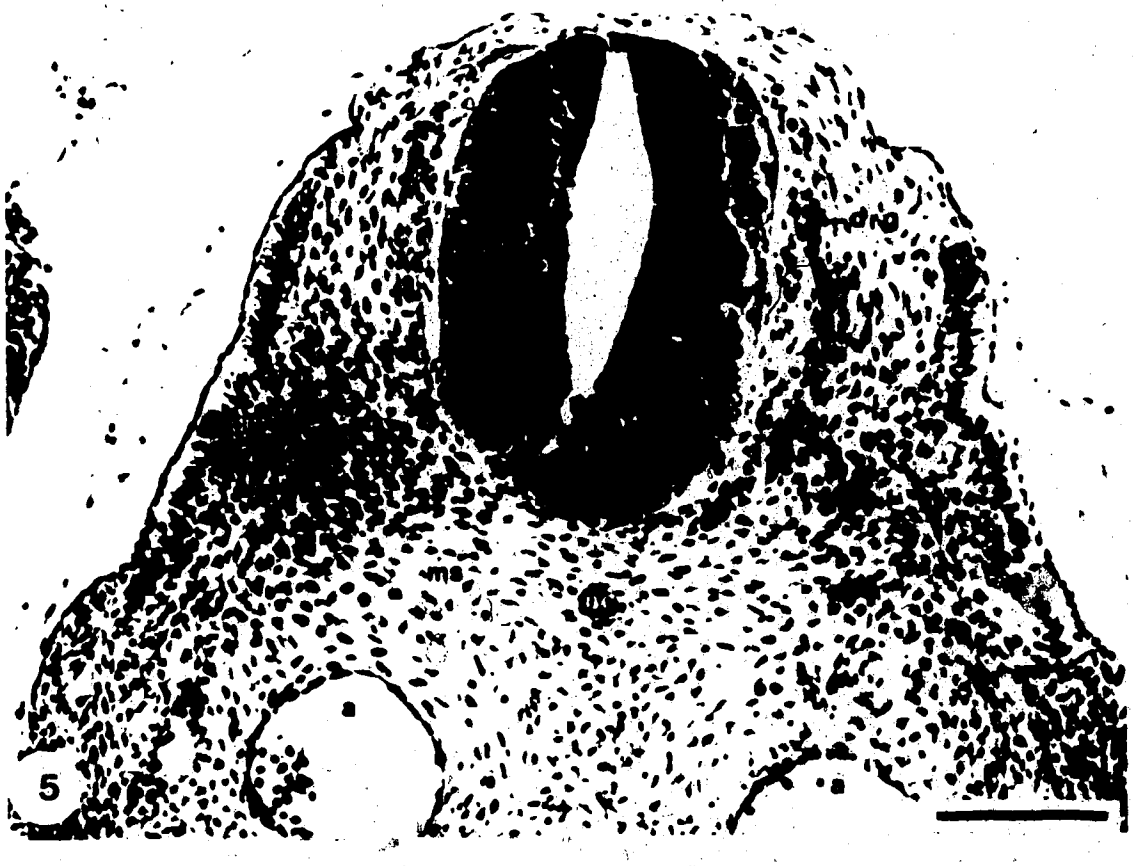


Figure 5
H86 3.5 mm Transverse Section
The neural tube has closed and the notochord is separated from the ventral aspect of the neural tube. The somites in this region are open and the sclerotome cells have extended to the notochord. The dermatomyotomes are present on the sides of the embryo, lateral to the neural tube. The cluster of cells adjacent to the neural tube on the right side of the figure represents the early formation of the dorsal root ganglion. Note the difference in the density of the cells around the notochord (the medial sclerotome) to that more laterally on the left side of the figure (the lateral sclerotome).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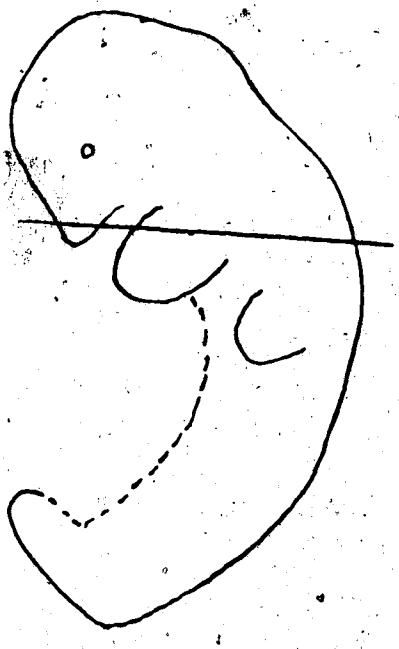

Figure 6
H86 3.5 mm Transverse Section
In the tip of the tail the neural tube is not well formed and remains unclosed, whereas it is closed in the cranial aspect of this embryo (compare to Fig. 5). Ventral to the neural tube, and closely applied to it, is the notochord. Ventrolateral to the notochord are two somites which have not yet broken down to release the sclerotome cells. A small blood vessel lies between the somites and the allantois in the ventral-most aspect of the tail.

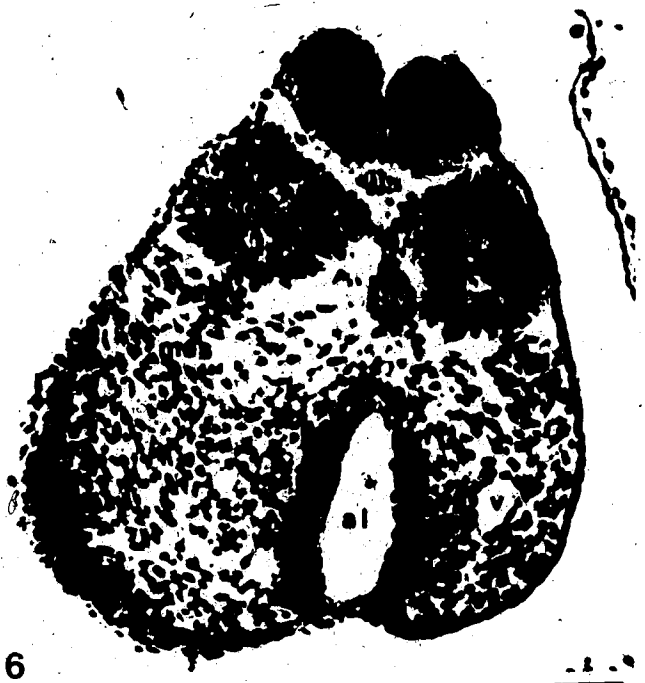

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Figure 7
Top view: H44 3.5 mm Coronal Section
Bottom view: Close-up of the area in the box
The somites, except those in the tail, are open and the cells have extended to the notochord. On the lateral edges of the embryo are the dermatomyotomes and developing kidney. Medial to them, reaching to the notochord and neural tube, are the sclerotome cells which retain the somitic segmentation with the elongated aggregations of blood cells. These blood cells reach the aorta and appear to be branches from it (arrows). The ventrodorsal extent of the blood cells is clearly indicated in this section. Due to the curvature of the embryo this section spans three levels: the aorta, which is just ventral to the sclerotomes, the notochord, which is in the approximate center of the sclerotomes, and the neural tube, which is dorsal to the sclerotomes. At all of these levels the intersclerotomal blood cells can be discerned, indicating that they extend throughout the majority of the ventrodorsal extent of each sclerotome.

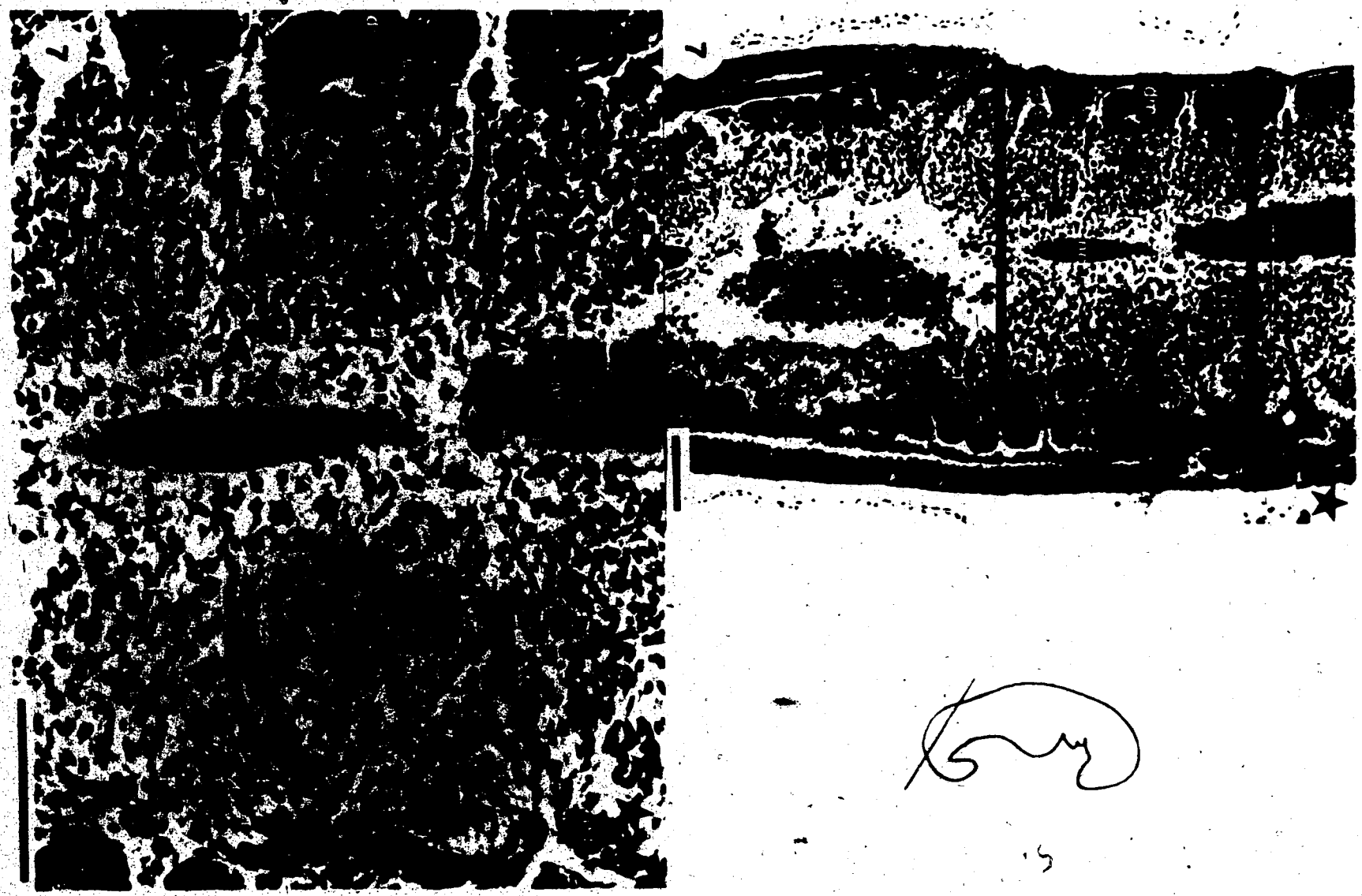
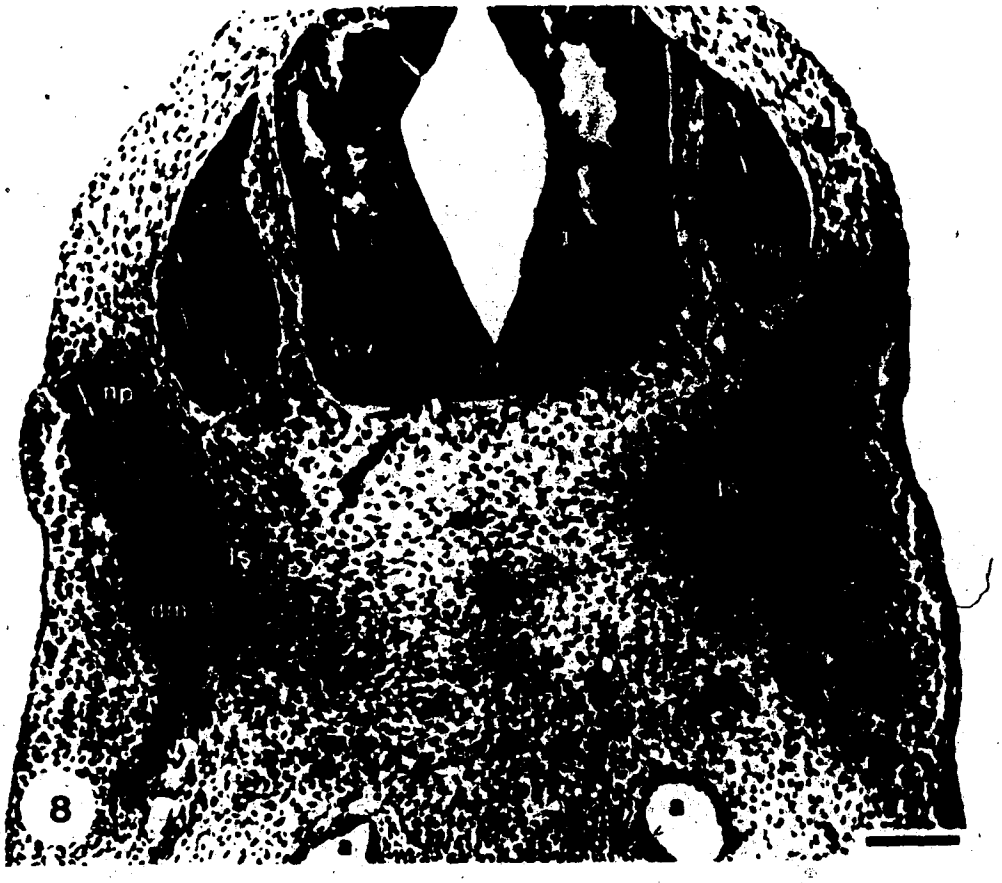


Figure 8
H42 6 mm Transverse Section
The neural tube and dorsal root ganglia are well developed (compare to Fig. 5). The neural processes are evident and extend dorsally between the dorsal root ganglia and dermatomyotomes. They originate from the dense lateral sclerotome.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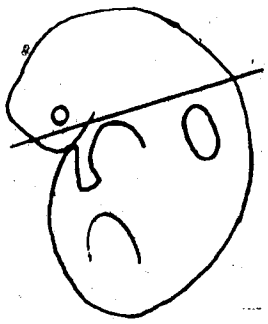

Figure 9
Top view: H42 6 mm Coronal Section
Bottom view: Close-up of area in box
The intersclerotomal blood cells extend opposite the cleft between adjacent dermatomyotomes to the notochord. Intrasclerotomal fissures extend opposite the middle of the dermatomyotome to approximately the medial edge of the lateral sclerotome. Directly cranial to each fissure on the lateral aspect of each segment are the spinal nerves. The oval structures at the lateral edge of the embryo on the left side of the figure are parts of the developing kidney.

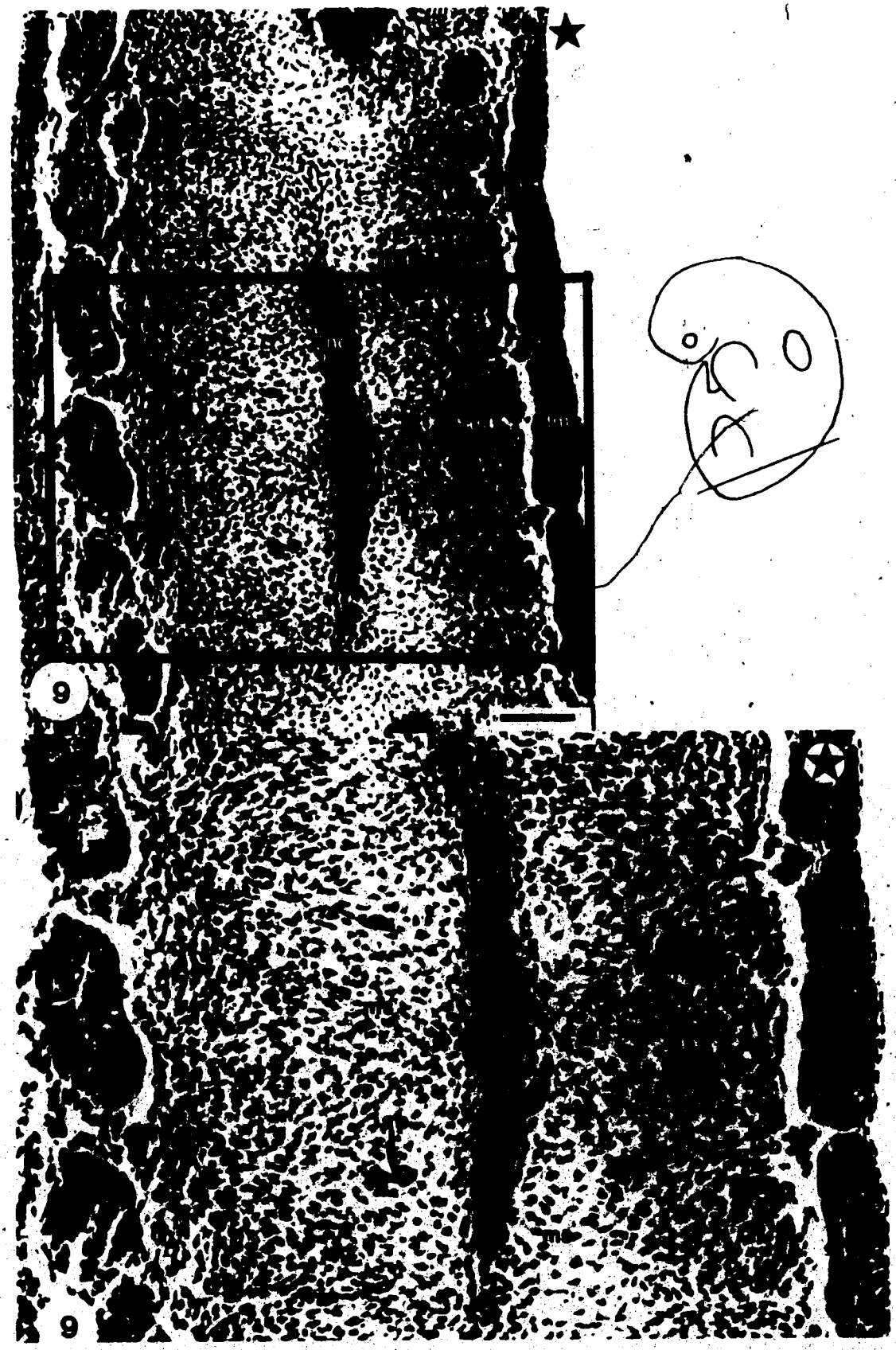
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Figure 10
H55 6 mm Coronal Section
The sclerotome is segmented by intersclerotomal arteries and intrasclerotomal fissures. The sclerotome cells cranial to the fissure and medial to the spinal nerve of each segment are lighter staining and form the light sclerotome band. The dark band is situated caudal to the fissure and is not associated with the spinal nerve. An artifactual tear has separated the dermatomyotomes from the lateral edge of the sclerotomes.

Figure 11
H66 6.5 mm Transverse Section
The sclerotome cells on the lateral aspect appear to be more dense than those around the notochord. From this lateral sclerotome arise the neural and costal processes. The neural processes are found throughout the entire vertebral column, whereas the costal processes (or ribs) are observed only in sections caudal to the level of the upper limb bud.

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nc
dp
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dm
11

Figure 12
H66 6.5 mm Coronal Section
The intrasclerotomal fissures extend from the medial edge of the dermatomyotomes to the notochord. Directly opposite the fissure is the myotomic bulge, a medial projection of the dermatomyotome. Due to the poor staining of this embryo, the dark and light bands cannot be distinguished clearly.

Figure 13
H28 7 mm Coronal Section
In this embryo, the dermatomyotomes have separated into their two components and the myotomes have joined to form a continuous column. More importantly, the bands of sclerotome have altered their position in relation to the arteries, fissures and spinal nerves. The light band now encompasses the intersclerotomal artery, whereas in less developed embryos it is caudal to the artery. The dark band now encompasses the intrasclerotomal fissure, whereas in less advanced embryos it is caudal to the fissure. The spinal nerve is now opposite the caudal half of the light band and the cranial half of the dark band, whereas in less developed embryos it is opposite the light band only.


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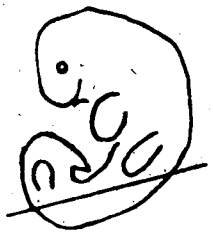


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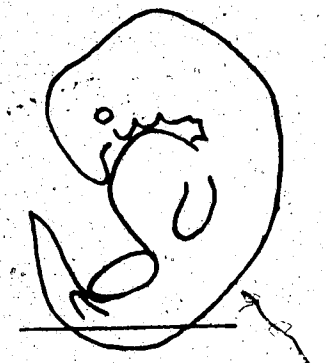
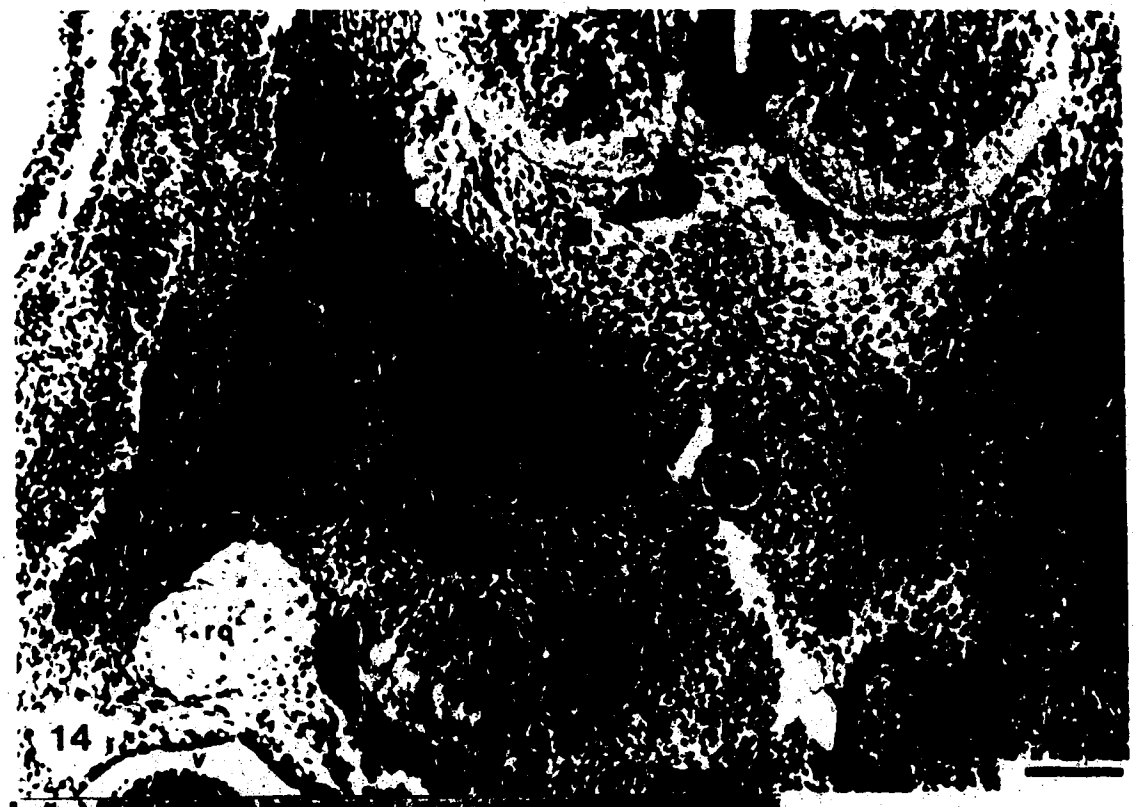


Figure. 14
H21 8.5 mm Transverse Section
The costal processes, or ribs, are observed only in transverse sections caudal to the brachial plexus. Note that the costal process is immediately lateral to the ramus communicans of the sympathetic trunk.

Figure 15
H21 8.5 mm Coronal Section
The relationship of the sclerotome bands is identical to that seen in H28 (compare to Fig. 13). The arteries are found in the center of the light band and the fissures in the dark band. The spinal nerves are still cranial to the fissures, but lie opposite parts of both the dark and light bands.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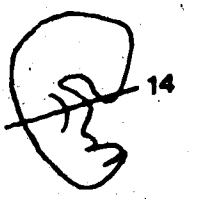
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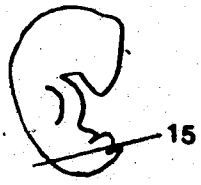
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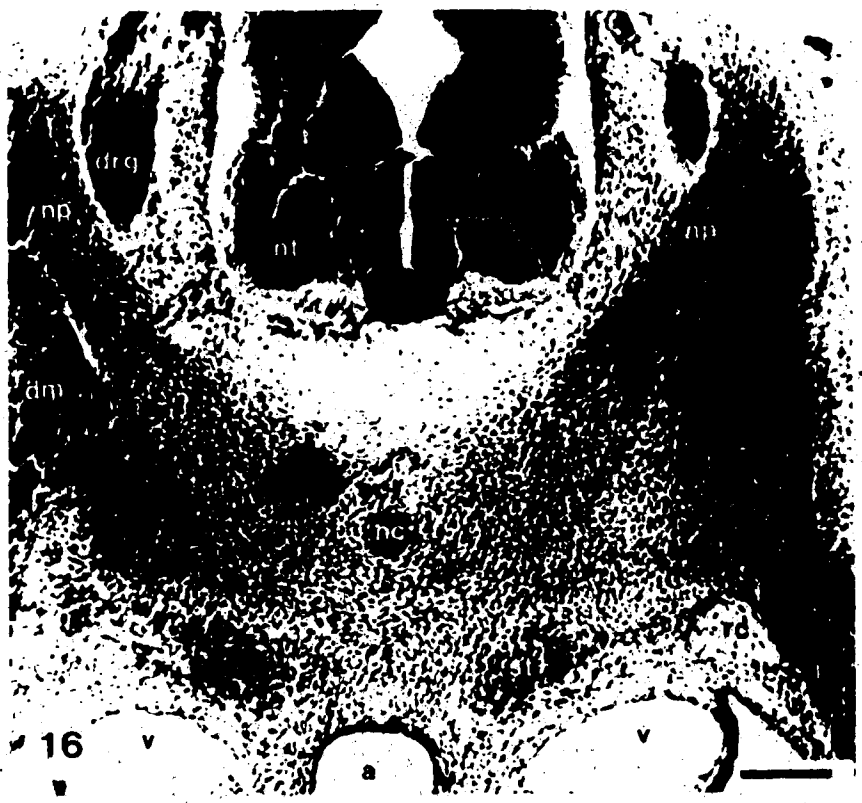
Figure 16
H58 9.4 mm Transverse Section
The dark sclerotome band is clearly visible in transverse sections and is united with the neural processes and ribs (compare to Fig. 17).

Figure 17
H58 9.4 mm Transverse Section
In contrast to the dark band (compare to Fig. 16) the light band, as shown here, has no processes associated with it. Note that the spinal nerves are positioned lateral to the sclerotome cells.



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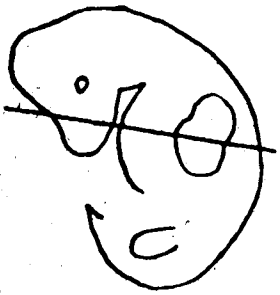

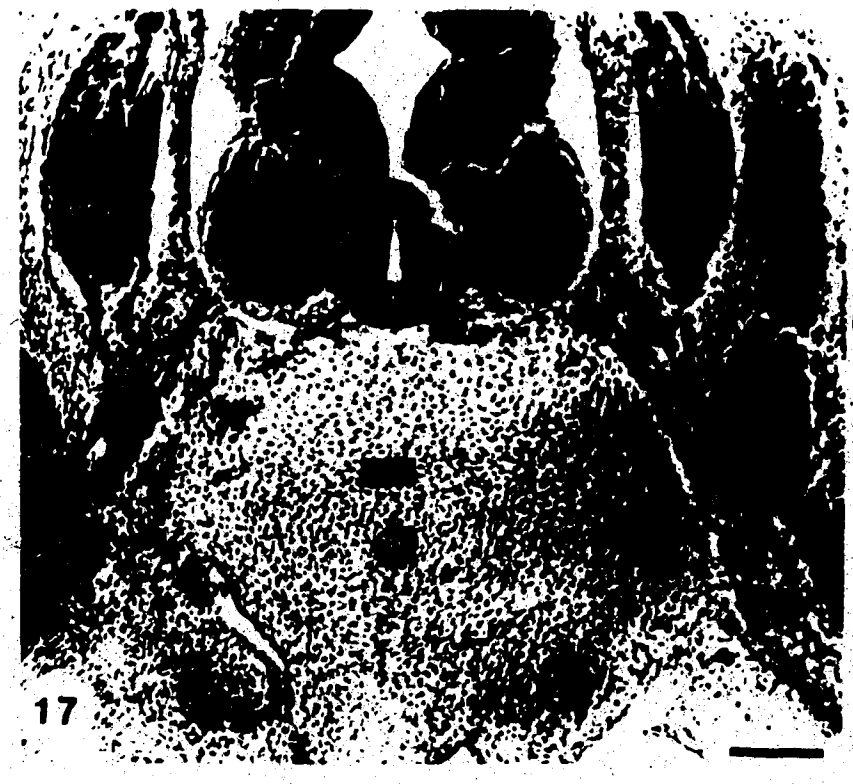

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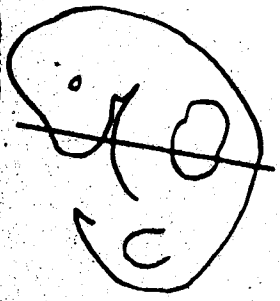

Figure 18
H58 9.4 mm Coronal Section
Part of the dark band appears to extend into the intermyotomic junction, forming the neural process. Note that the distinction between the medial and lateral sclerotomes is no longer visible. Also note the mediolateral extent of the intrasclerotomal fissures.

Figure 19
H34 10 mm Coronal Section
The neural processes are more clearly developed in this embryo than in H58 (compare to Fig. 18). They arise from the lateral part of the dark band caudal to the spinal nerve and extend between adjacent myotomes.

18

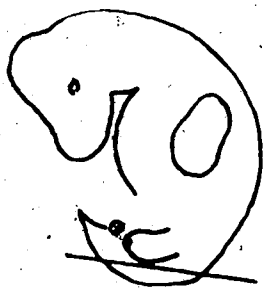

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Figure 20
H45 11 mm Transverse Section
In the light sclerotome band, as shown here, there is occasionally a band of cells extending from the notochord to both the dorsal and ventral edges of the sclerotome. (The ventral extension of this band is not visible due to an artifactual tear). This is not a consistent feature in this embryo.

Figure 21
H45 11 mm Transverse Section
This section indicates the position of the spinal nerve medial to the shaft of the rib.

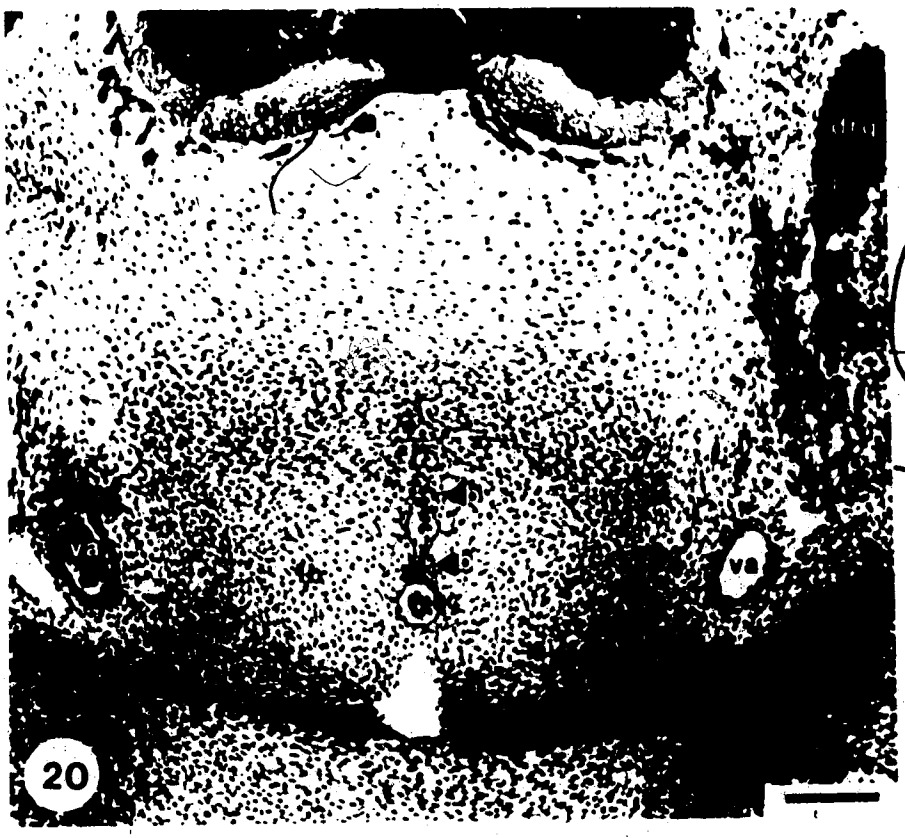
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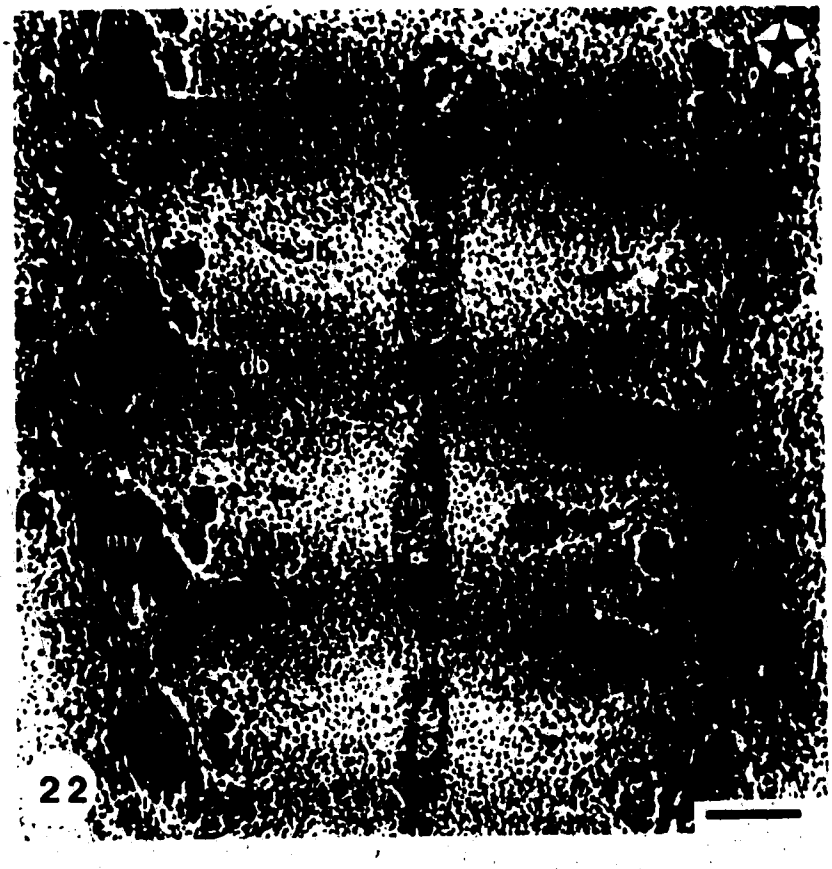
Figure 22
H45 11 mm Coronal Section
This section shows the relationship of the spinal nerve to the dark and light bands, which is identical to that of H28 (compare to Fig. 13). The spinal nerve is opposite the caudal-most aspect of the light band and the cranial-most aspect of the dark band. The intersclerotomal arteries and intrasclerotomal fissures are also visible.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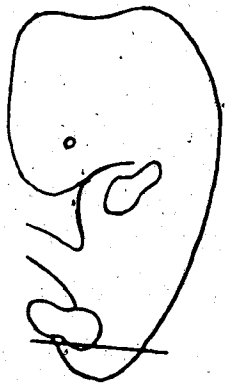
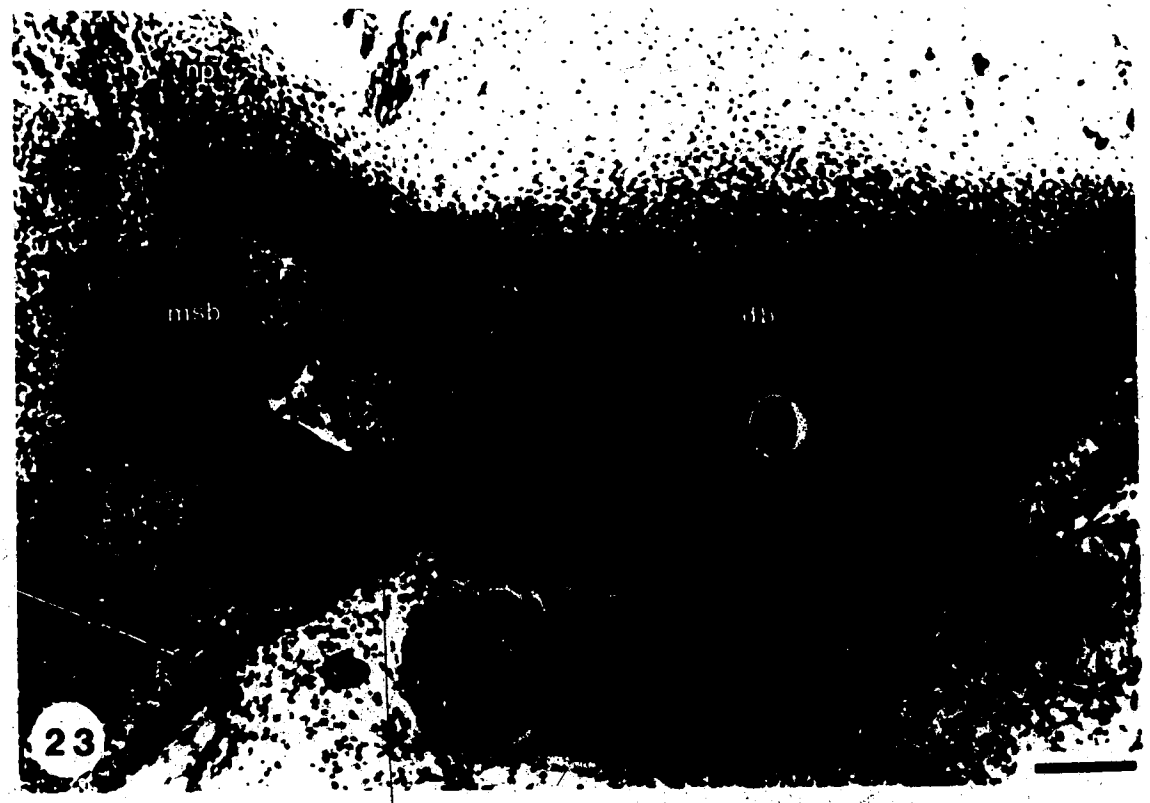


Figure 23
H43 12.5 mm Transverse Section
The head of the rib is almost a separate entity and between it and the remaining sclerotome cells is a blood vessel. Joining the neural process and rib head is a mesenchymal band which is the anlage of the thoracic transverse process.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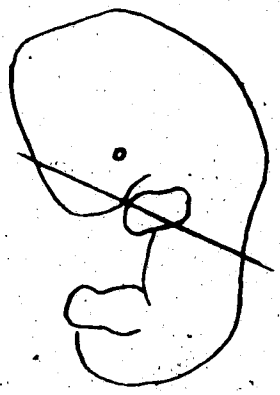
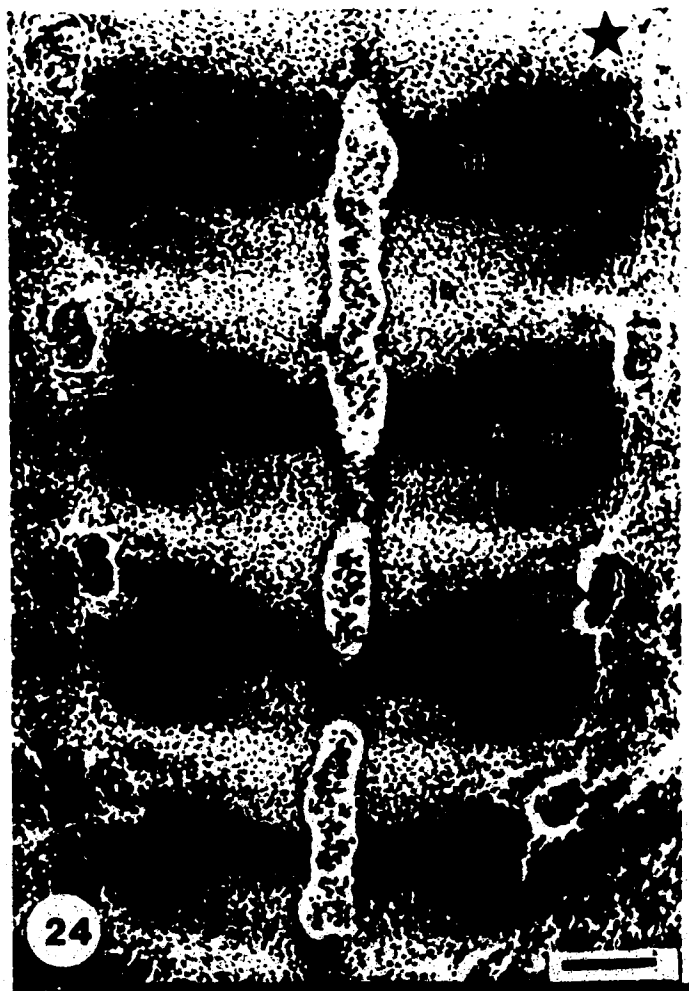


Figure 24
H43 12.5 mm Coronal Section
The intersclerotomal arteries and intrasclerotomal fissures are no longer visible. The spinal nerve is opposite the caudal half of the light band and the cranial aspect of the dark band. The dark band is not uniform in density, but is divided into two regions: zone A, the upper more dense area and zone B, the area of less density just caudal to zone A. Note that zone A extends below the level of the spinal nerve.

Figure 25
H57 14 mm Coronal Section
The coronal sections of this embryo are similar to those of H43 (compare to Fig. 24). Zone A is dense and unchondrified whereas zone B is lighter and is undergoing chondrification. The light band is also chondrifying.



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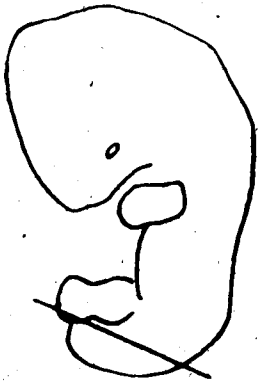

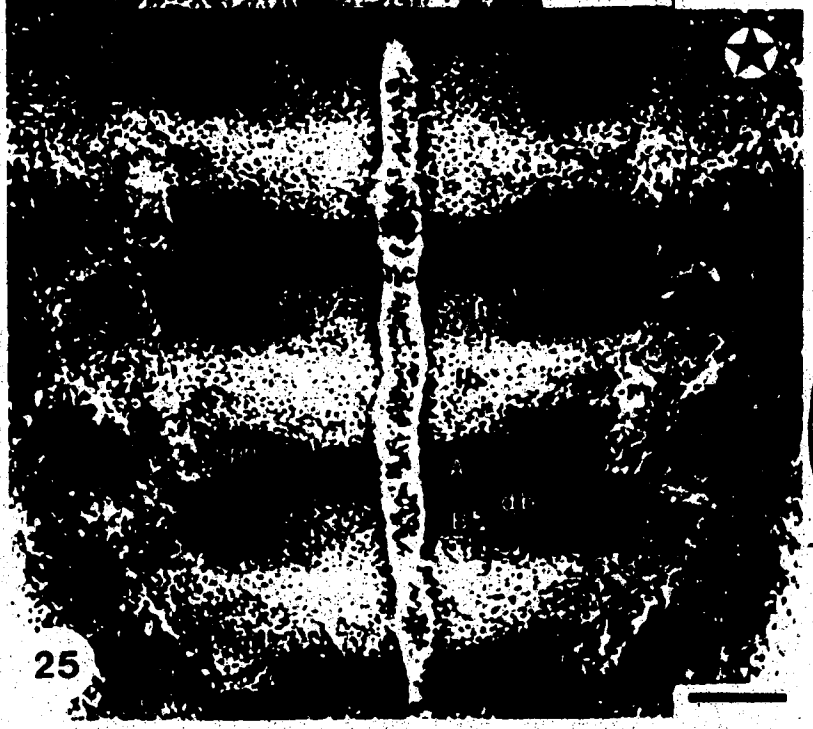

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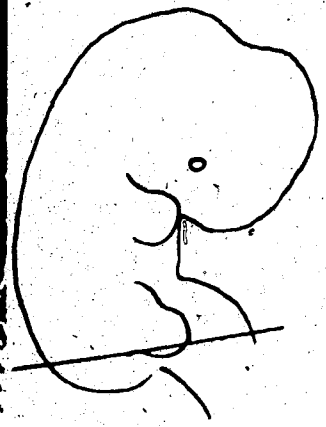
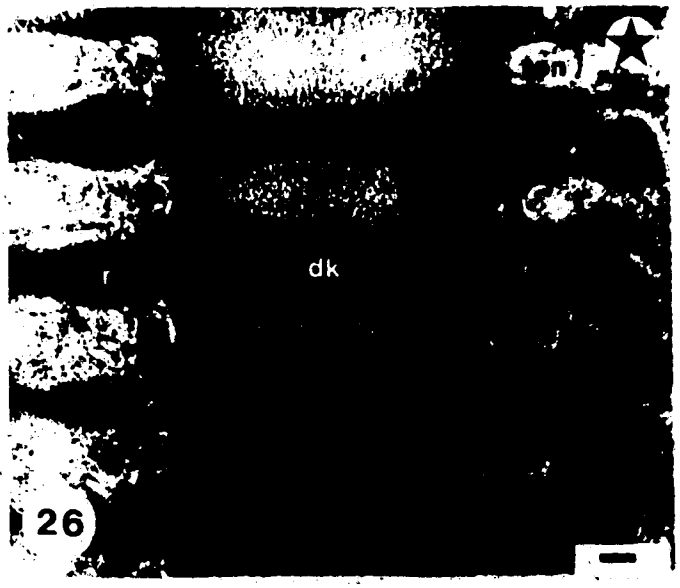


Figure 26
H19 14.6 mm Coronal Section
The vertebral bodies and ribs, which are undergoing chondrification, and the intervertebral disks are shown here. Note the position of the vessels on either side of the vertebral body and the spinal nerves between the ribs.

Figure 27
H19 14.6 mm Coronal Section
The ribs and articulating processes are both shown in this section. A mesenchymal band joins the articulating processes and the inferior articulating processes appear to be longer than the superior ones.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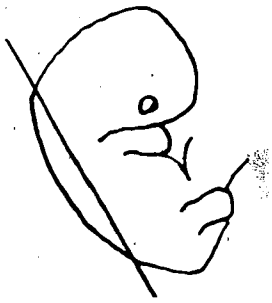

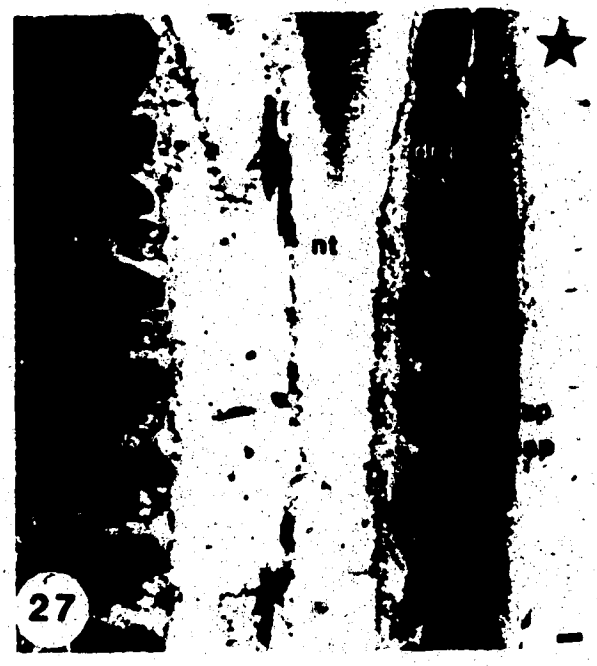
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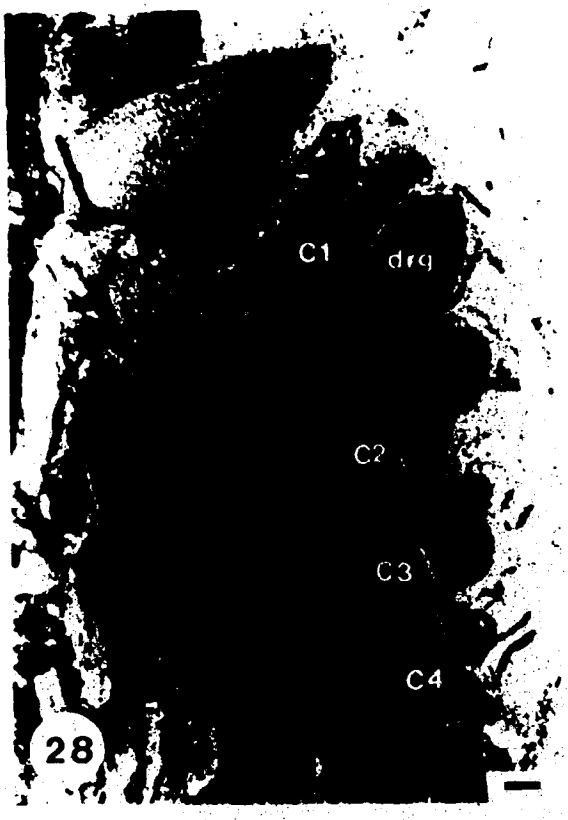
Figure 28
H109 12 mm Sagittal Section
The suboccipital nerve is located between the occiput and the first cervical vertebra; it is not associated with a dorsal root ganglion. The vertebral artery is cranial to the nerve.

Figure 29
H109 12 mm Sagittal Section
The first dorsal root ganglion is between the first and second cervical vertebrae. Note that the first rib is opposite the seventh cervical vertebra in this embryo. Also note the large spinal nerves ventral to the cervical vertebrae.


C1
drq
C2
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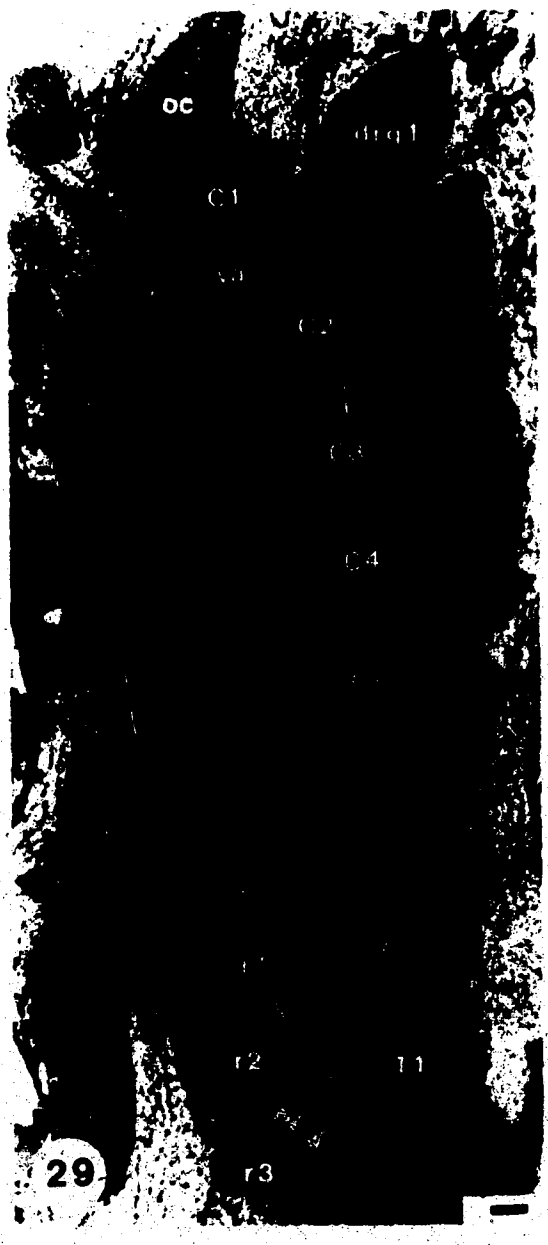
C4
28


oc
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C4
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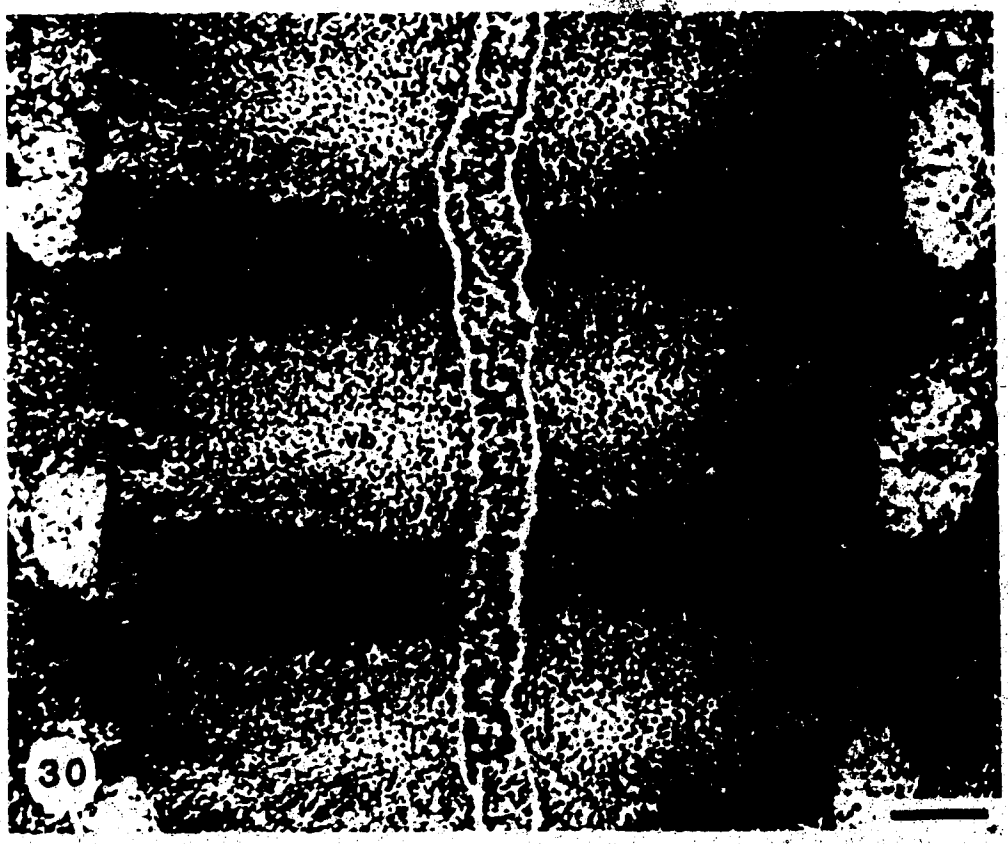
29

Figure 30
H60 15 mm Coronal Section
The vertebral bodies are undergoing chondrification which distinguishes them from the unchondrified intervertebral disks. The cranial half of the disk is opposite the lower aspect of the spinal nerve and the disk extends a short distance below the level of the nerve.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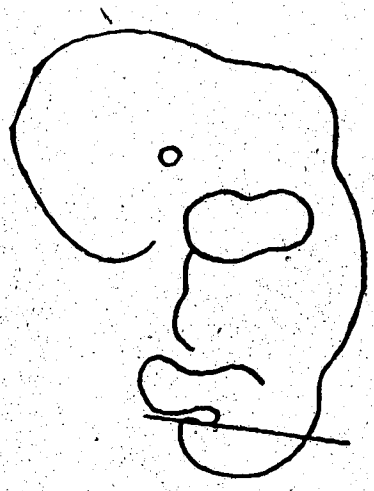
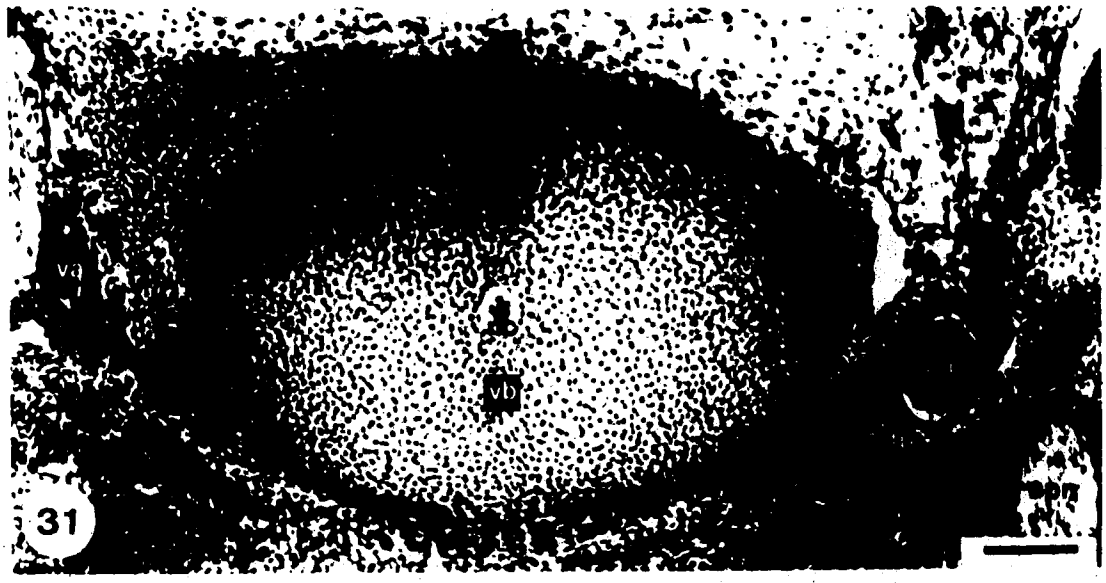


Figure 31
H80 17.5 mm Transverse Section
The cervical vertebrae have typical transverse processes which surround the vertebral artery. Parts of the spinal nerves are located lateral to the transverse processes in this section.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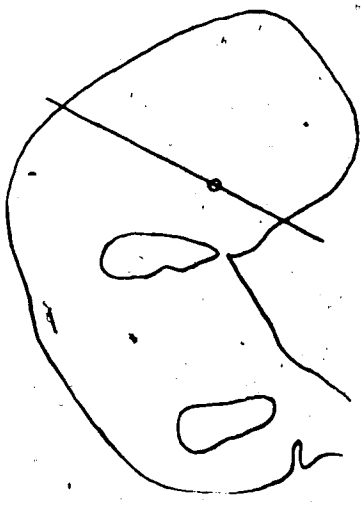
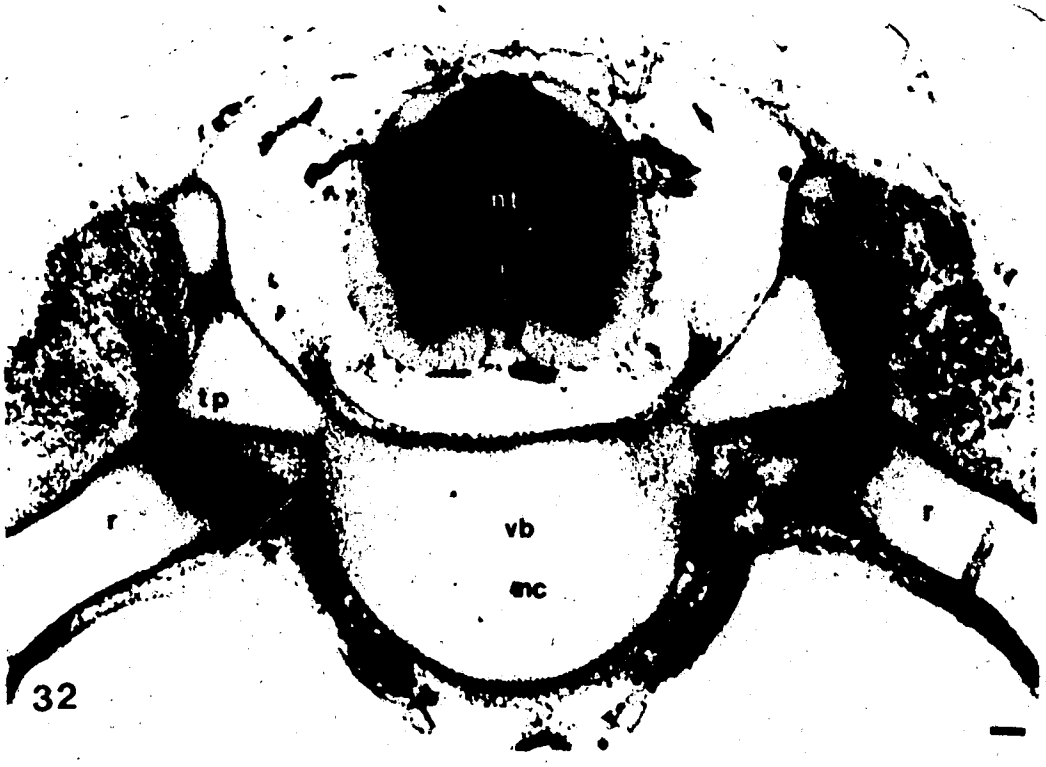


Figure 32
H51 15.5 mm Transverse Section
The thoracic vertebrae possess cartilaginous transverse processes which emerge from the neural processes to articulate with the head of the rib. Note the relative smallness of the neural tube in comparison to the size of the vertebrae.



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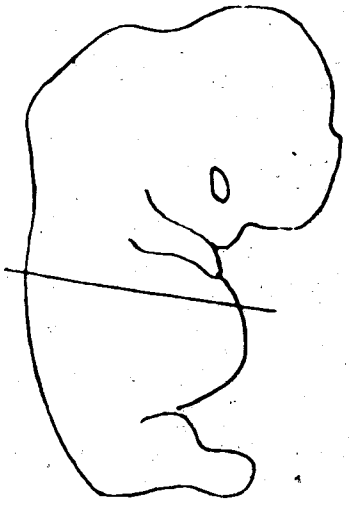
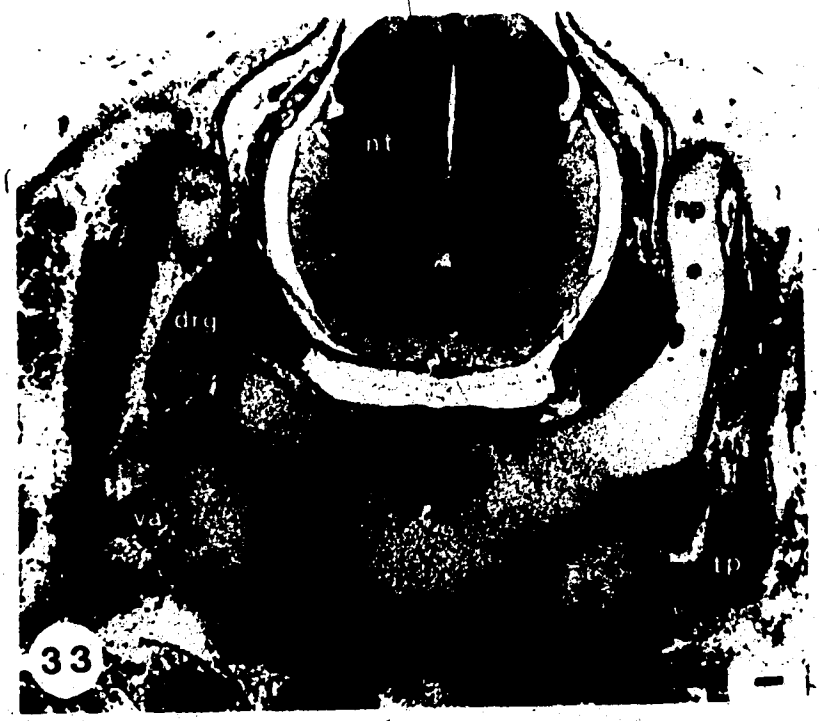


Figure 33
H81 23.5 mm Transverse Section
The ventral arch of the atlas vertebra is situated ventral to the axis vertebra. Note the absence of the vertebral body in the atlas vertebra and the transverse processes partially surrounding the vertebral arteries.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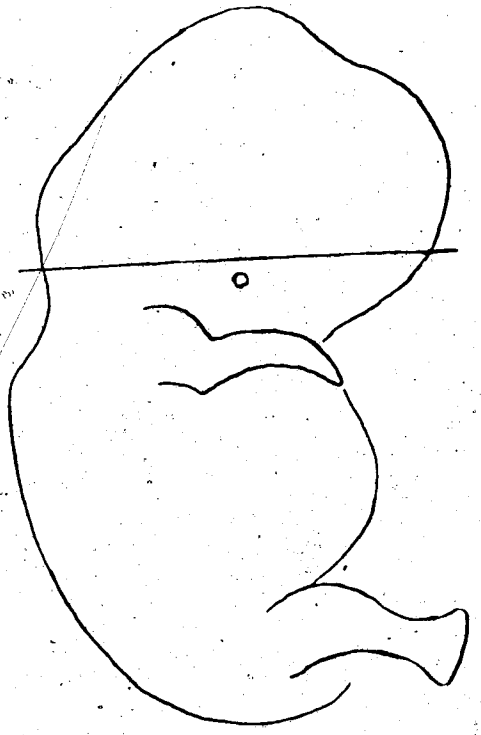

Figure 34
H81 23.5 mm Transverse Section
The ventral arch of the atlas vertebra is ventral to the odontoid process of the axis. Note the presence of the notochord in the odontoid process and the mesenchymal anlage of the transverse ligament. Also note the trilobed appearance of the odontoid process.


drg
34
bv

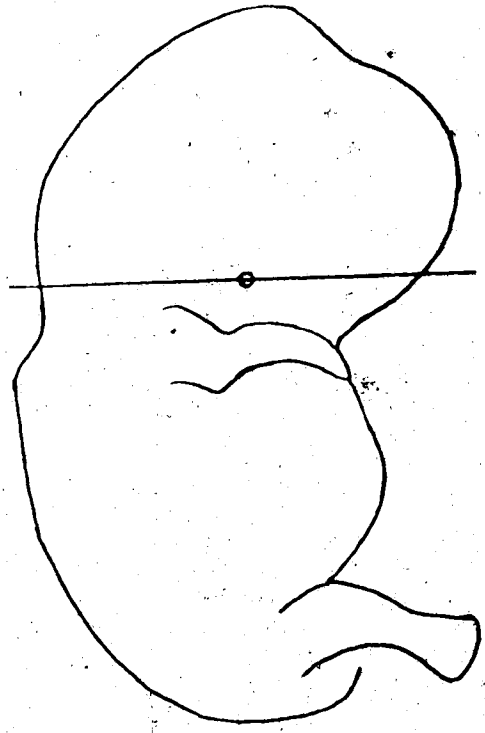

Figure 35
H81 23.5 mm Coronal Section
The lateral-most aspects of the sacral vertebrae are fusing. However, the bodies of the sacral vertebra are separated by intervertebral disks.


dk
vb
hc
35

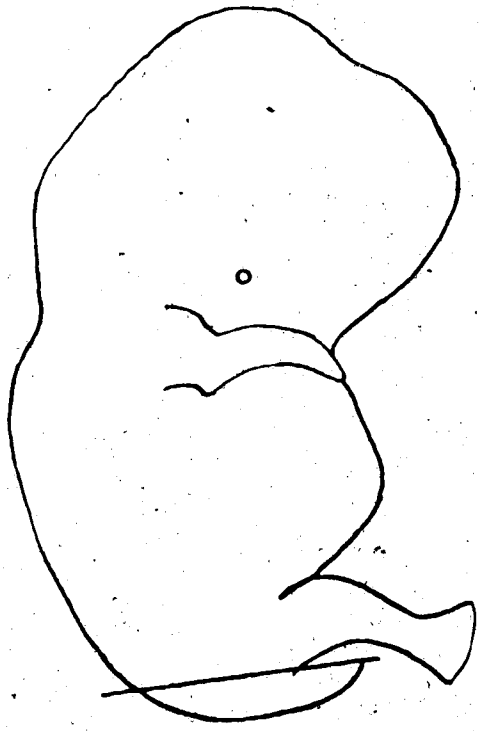

Figure 36
H104 22 mm Sagittal Section
The vertebral artery is in a position similar to that found in the adult: it arches over the seventh cervical vertebra and passes through the transverse processes of the upper six cervical vertebrae. Note the wedge-shaped anterior arch of the atlas and the muscle tissue between the transverse processes.

Figure 37
H104 22 mm Sagittal Section
Between each rib in the upper part of the intercostal space is a neurovascular bundle. Muscle tissue is also found in the intercostal spaces.

36

37

Figure 38
H104 22 mm Sagittal Section
The bodies of the lumbar and sacral vertebrae are shown here. While the lumbar vertebrae are clearly separated by intervertebral disks, the sacral vertebrae are fusing together. Compare the sacral vertebrae in this section to those of figure 35 in which the vertebral bodies are separated by intervertebral disks.

L
Vb
38

Figure 39

A-D are diagrammatic representations of the process of vertebral development as described in the current study.

A. Each sclerotome segment (the cranial-most segment is designated by brackets throughout these diagrams) is bounded by intersclerotomal arteries and is divided into two halves by an intrasclerotomal fissure. Each sclerotome half is of equal density. The spinal nerve is positioned cranial to the intrasclerotomal fissure and opposite the cranial sclerotome half.

B. The caudal sclerotome half becomes darker than the cranial half and this dense area forms the dark sclerotome band (stippled area). The light sclerotome band occupies the cranial sclerotome half and is opposite the spinal nerve. The intrasclerotomal fissure separates the two bands of each segment.

C. A relative change in the positions of the sclerotome bands occurs. The light band surrounds the intersclerotomal artery while the dark band surrounds the intrasclerotomal fissure. The spinal nerve is now opposite the caudal aspect of the light band and the cranial aspect of the dark band, while remaining cranial to the intrasclerotomal fissure as in diagrams A and B. The dark band soon develops two densities within it: a very dense zone A and a less dense zone B. Concurrently, the intrasclerotomal fissures and intersclerotomal arteries are no longer visible in the sections (they have not been removed from the diagram). Since zone A extends below the level of the spinal nerve (the caudal-most level of zone A is indicated by arrows), if the fissures were present (as indicated on the diagram) this dense area would encompass the fissure. Zone B extends from the level of the arrow to the caudal aspect of the neural processes. The neural processes and costal processes are both initially outgrowths of zone B of the dark band.

D. This diagram illustrates the cartilaginous vertebral bodies and intervertebral disks. (The contribution of one sclerotome segment to the vertebral bodies and disks is indicated by the bracket). Zone A of the dark sclerotome band forms the intervertebral disk while zone B and the light band caudal to it chondrify to form the vertebral body. The spinal nerve is opposite the caudal aspect of the vertebral body and the subjacent intervertebral disk. Ultimately, one segment contributes to one intervertebral disk and parts of two vertebral bodies: the caudal aspect of the vertebral body cranial to the disk and the cranial aspect of the vertebral body caudal to the disk.

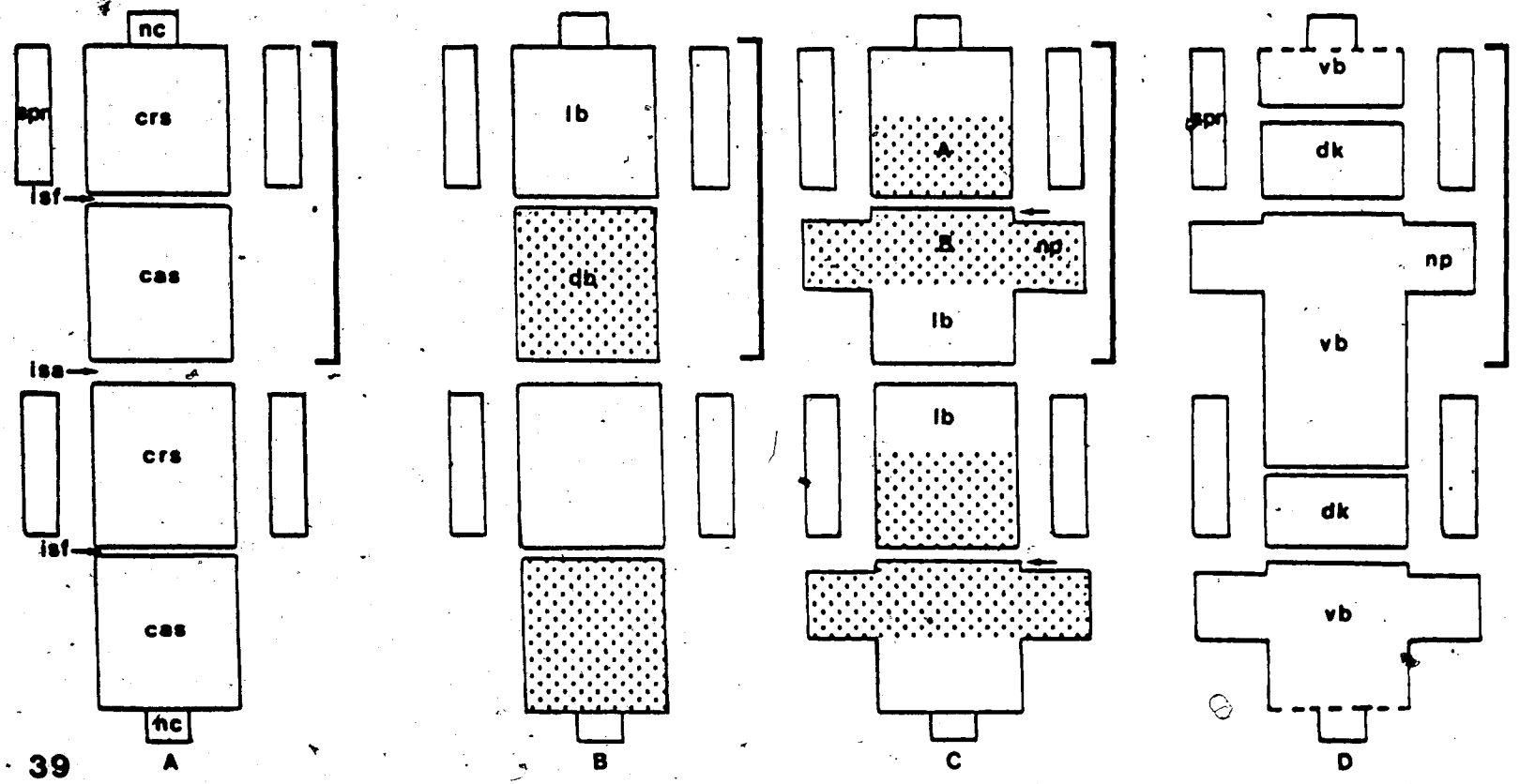
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Figure 40

A-C are diagrammatic representations of various theories of human vertebral development. The two blocks at the left represent somites and the original segments of the vertebral column. The dark bands are indicated with hatching. Only the neural processes are shown in the diagrams.

A. Human vertebral development according to Bardeen and Lewis (1901).

1. The caudal third of each segment is dense and the neural and costal processes emerge from it. (The intrasclerotomal fissures are not described and are therefore not included in these diagrams).

2. The dense caudal third contributes to the intervertebral disk in addition to the vertebral body caudal to the disk. The majority of the vertebral body is composed of the light band.

B. Human vertebral development according to Bardeen (1905) and Ehrenhaft (1943).

1. Initially the caudal sclerotome half is more dense than the cranial one. (The precise position of the neural processes is not clear in either theory and therefore they are not included in these diagrams).

2. The position of the dense zone changes and it now surrounds the intrasclerotomal fissure.

3. The intervertebral disk develops from the dense area surrounding the intrasclerotomal fissure. The light areas of two adjacent segments form the vertebral body.

C. Human vertebral development according to Wyburn (1944).

1. Initially the dense area is confined to the caudal sclerotome half. (The precise position of the neural processes is not clear and therefore they are not included in these diagrams).

2. The dense area is reinforced by cells of the cranial sclerotome half and it now surrounds the intrasclerotomal fissure.

3. Each dense area is divided into three zones: a very dense central zone C and two less dense zones, A and B, cranial and caudal to it. (The relationship of the intrasclerotomal fissure to these three zones is not clearly described and therefore the fissure is not shown)

4. Zone C forms the intervertebral disk. The vertebral body is a composite structure consisting of the light band plus zone B cranial to it and zone A caudal to it.

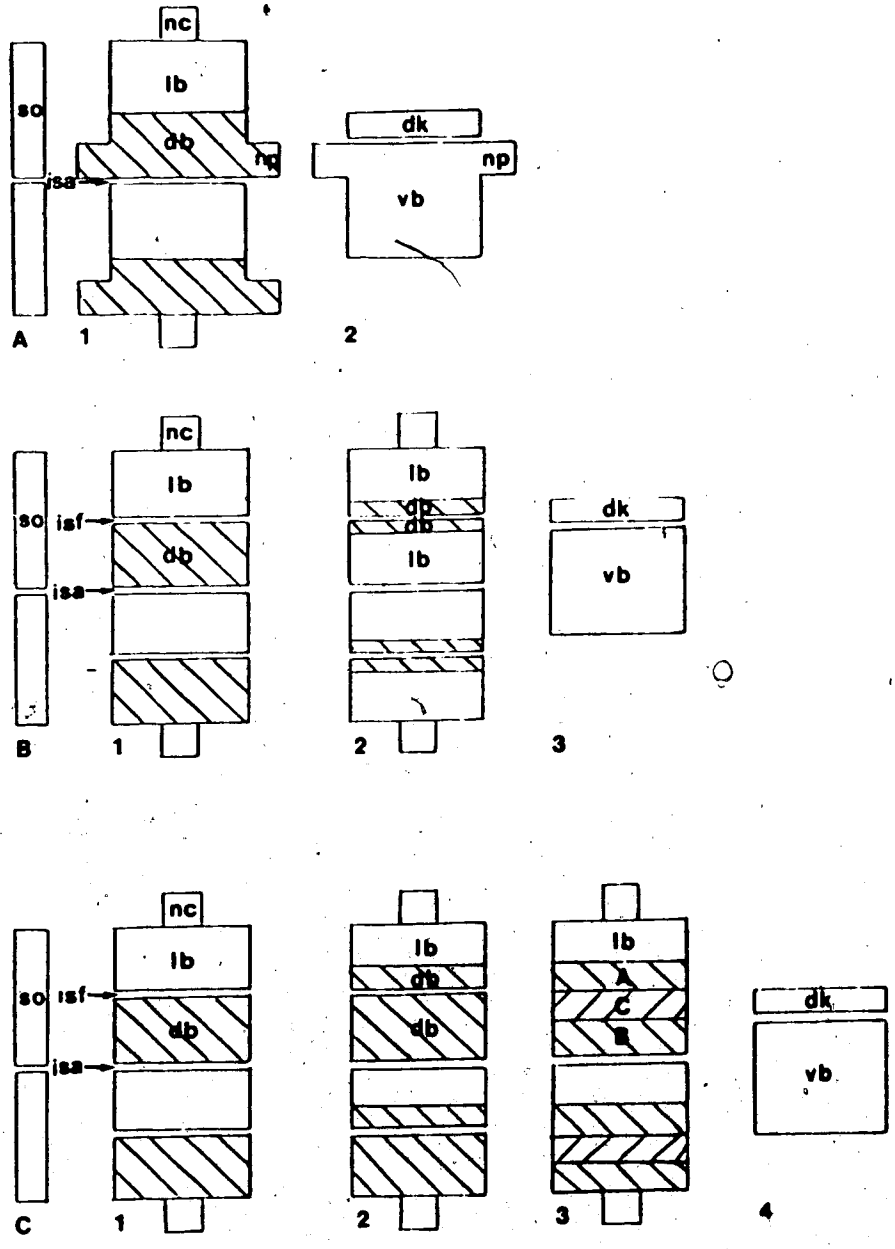
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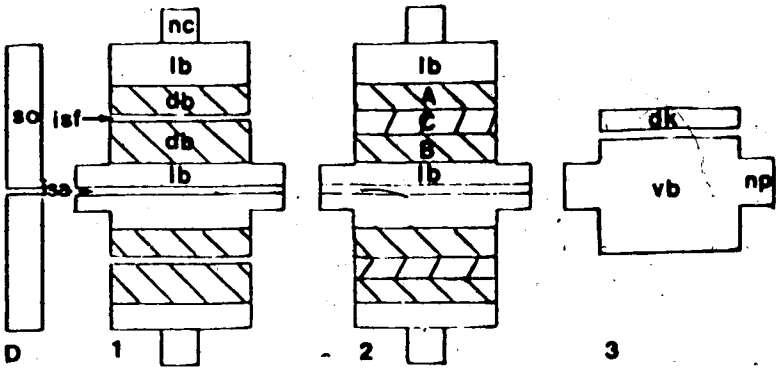
Figure 41
D-F are diagrammatic representations of the process of human vertebral development (continued from Figure 40).
D. Human vertebral development according to Sensenig (1949).
1. The dense area surrounds the intrasclerotomal fissure. The neural and costal processes are intersegmental structures originating from two segments.
2. Each dense area is divided into three zones: a very dense central zone C and two less dense zones, A and B, cranial and caudal to it. (The relationship of these zones to the intrasclerotomal fissure is not clearly detailed and therefore the fissure is not shown).
3. The central zone C forms the intervertebral disk. Each vertebral body is formed from the light band plus zone B cranial to it and zone A caudal to it.
E. Human vertebral development according to Sensenig (1957).
1. The caudal sclerotome half is more dense than the cranial one. The neural and costal processes are intersegmental structures.
2. The intervertebral disk forms from part of the dense zone. The vertebral body forms from the light areas with additions from the dark bands cranial and caudal to it.
F. Human vertebral development according to Peacock (1951).
1. The caudal sclerotome half is more dense than the cranial one. (No details of the neural processes were given and therefore they are not shown).
2. The position of the dark band has changed and it now surrounds the intrasclerotomal fissure.
3. The intervertebral disk develops from the dense zone. The vertebral bodies are intersegemental structures, arising from the light areas of two adjacent segments.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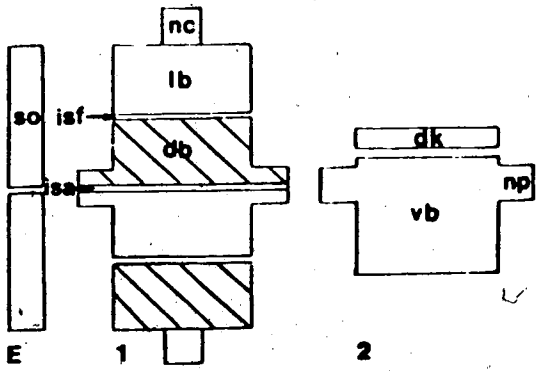
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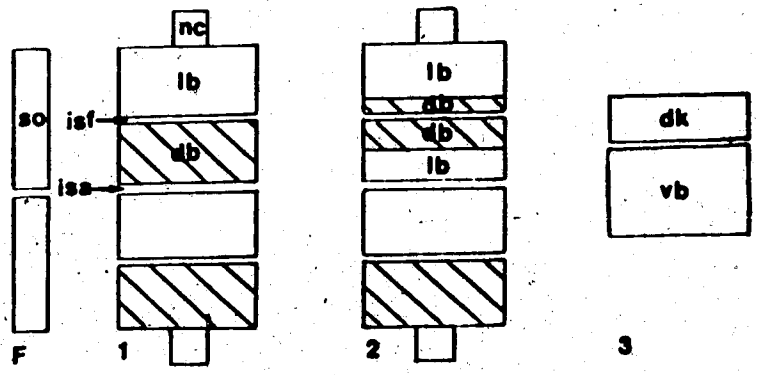
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IX. APPENDIX

A. Techniques of Serial Reconstruction

A Wax Reconstruction of H104

A wax model was reconstructed from the left half of the first five thoracic vertebrae of H104 using plates of dental wax. Only the vertebrae, but not the ribs, were included in the reconstruction.

1. The dental wax was measured with micrometer calipers and found to have an average thickness of 1500 μm per plate. Since the embryonic sections were cut at 10 μm, and every second section was to be reconstructed, the sections were magnified to 75x in order to maintain the correct proportions in the model.

2. The sections were projected with a Leitz Prado microscope projector onto sheets of paper pinned onto the wall.

3. The outlines of the vertebrae were traced in pencil onto the sheets of paper.

4. Each tracing was placed on a light box and a plate of wax superimposed on top of the paper. The outlines were retraced onto the wax plates.

5. Once all of the outlines were traced, the features were cut out of the wax plates with a sharp scalpel. Bridges of wax were left between separate elements to maintain their correct relationships.

6. The wax outlines were then lightly scraped with a razor blade to remove the raised wax edges.

7. The outlines traced onto paper were stacked serially, using the best fit method, and were then used to guide the alignment of the wax plates. White glue was used to bond the wax plates together.

8. The excess glue was scraped off and heated wires were placed into the model in areas which needed support. After this step was completed, the bridges were removed with a heated metal spatula.

A Transparent Graphical Model of H104

The first five thoracic vertebrae of H104 were reconstructed again with the use of acetate sheets. The vertebrae, ribs, nervous tissue, blood vessels and, as ancillary structures for the use of alignment, the lungs were traced.

1. Two thicknesses of acetate were available, 5 mil (125 μm) and 10 mil (250 μm), which had a combined average thickness of 375 μm. The embryonic sections were cut at 10 μm. Therefore, utilizing every section, a magnification of 37.5x was used to maintain the proportions of the model.

2. The sections were projected down onto sheets of acetate using a Bausch and Lomb microscope projector.

3. The structures of interest were traced onto the acetate, using permanent, fine-tipped felt pens. A different color was used for most structures.

4. A cardboard sheet with a series of small holes was used to aid in the alignment of the tracings. The first tracing was placed onto a lightbox and securely taped in place. The cardboard sheet was placed on the tracing and the outlines of the holes were drawn onto the first sheet. These circles were placed on the periphery of the sheet, outside of the tracings so they would not interfere with the model. A piece of 10 mil acetate, which was used as a spacer to maintain the correct proportions of the model, was placed on top of the tracing and taped in place.

5. The next tracing was aligned in accordance with the first, using the outlines of the lungs and other structures. When an alignment had been reached, the sheet was carefully taped onto the lightbox and the circles drawn on the first sheet were retraced onto the second sheet. In order to accomplish this, the cardboard sheet was used and the holes lined up with the circles on the first sheet. The circles could then be easily and accurately traced onto the second sheet. When the structures became larger or shifted so that the reference circles were included within the outlines, new ones were produced, again on the periphery of the sheet. A 10 mil sheet was then placed on top of the second tracing and taped onto the light box.

6. This process continued until all of the tracings were aligned. Since the acetate sheets had a bluish tinge to them, only ten or fifteen sheets could be stacked on top of each other before the stack was no longer transparent. When

this stage was reached, the bottom sheets were removed, and the top two or three sheets were aligned and taped onto the light box and the process of aligment was begun again.

Transparent Graphical Models of H42, H44 and H86

Using the process outlined above for the production of a graphical reconstruction of H104, graphical reconstructions of three more embryos were attempted. However, Repro-Tran Xerographic Transparency Film sheets were used rather than acetate sheets. The Repro-Tran sheets were chosen as they did not have to be cut into sheets as did the acetate and the surface of the sheets was slightly rough so that tracing with felt pens was easier. All of the sections were projected using a Leitz Prado microscope projector.

In order to obtain a very large and detailed view of the coronal tail sections of H42, a magnification of 610x was chosen. Part of each section, which included the neural tube, notochord, sclerotome and dermatomyotomes, was traced onto two transparency sheets which had been taped together. Only ten sections were reconstructed because of the problems outlined in the Materials and Methods section concerning the tracing of loose mesenchymal masses.

H44 was reconstructed in a similar manner as H42, except that the entire outline of the coronal sections of the rump were traced. This included blood vessels, nervous tissue, the dermatomyotomes and the sclerotome. A

magnification of 121x was chosen. All of the coronal sections, seventeen in all, other than the very dorsal-most sections which contained little vertebral material, were traced.

Another attempt at making graphical models on transparency sheets was attempted with H86. Rather than reconstructing coronal sections, a number of transverse sections were traced. The sclerotome in this embryo was not well condensed and led to problems with tracing the outlines of the sclerotomes. Several methods of shading and outlining the sclerotomes were attempted and the most satisfatory was found to be simple outlining of the densities. Thirty-one sections, beginning in the pharyngeal region, were projected with a Leitz Prado microscope projector at a magnification of 212x and traced. During this process, the subjectivity of the boundaries of the loose sclerotome came into question. To test this, one section was redrawn on five different days. The outlines were found to be substantially varied in these five tracings.

A Computer Model of H89

Although no attempt was made to produce a computer model, one such model had been created by others at an earlier date. Twenty-four sections of H89, a 44 mm embryo from the Shaner collection, were reproduced and serially aligned on a Hewlett Packard 9845B.

A Contour Model of H104

One contour model was reconstructed from the tracings made of the first five thoracic vertebrae of H104, which had been used in the construction of a wax model.

1. The original tracings (at 75x magnification) were aligned and three reference marks drawn onto each sheet.

2. A master sheet of paper was placed on a lightbox and the reference marks were traced onto it.

3. Each tracing was then retraced in pencil onto the master sheet in accordance to the reference marks.

4. Twenty outlines were reconstructed in this fashion. No attempt was made to shade or introduce perspective to the model.

A Photographic Reconstruction of H58

A photographic model was made with the coronal sections of H58. This method was chosen as it did not involve tracing poorly condensed sclerotome, as did the other methods of reconstruction. Therefore, there was no subjectivity in the production of this model.

1. Thirty-five coronal sections of the caudal region of the embryo were photographed in black and white Kodak Panatomic X film on a Leitz orthoplan photomicroscope.

2. The sections were enlarged to a magnification of 90x and printed. Since the photographic paper was not transparent, it was not necessary to adjust the magnification of the sections to that of the thickness of

the photographic paper.

Two more efforts were made to convert the photographs into a three-dimensional model. The first was to photocopy the photographs, which resulted in a representation of the sections on transparent sheets. The second was to trace the photographs onto transparency sheets using the methods used in the construction of graphical models. Both methods were unsatisfactory; the photocopies has poor contrast and many of the details were lost and the mesenchyme was not sufficiently condensed to render reliable tracings.