Acta orthop. scand. 52, 331–338, 1981

# SIGNIFICANCE OF RESEGMENTATION IN THE PATHOGENESIS OF VERTEBRAL BODY MALFORMATION

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Human embryos and fetuses were investigated histologically to elucidate the possible pathogenesis of congenital malformations of the vertebral body. Special attention was paid to the early development of the vertebral column, particularly the often-questioned stage of resegmentation, important as the stage during which the malformations might develop. Evidence of resegmentation was found in 7 embryos ranging from 7 mm C-R length (5 weeks after fertilization) to 13 mm (6 weeks). Moreover, the formation of the definitive vertebral body was considered to be intimately related to the intersegmental artery. As for Junghanns' concept that the cartilaginous body consists of two lateral halves, it was not observed in our specimens, nor was it found in the latest literature on normal vertebral development. Congenital malformations of the vertebral body were classified into two categories: failure of formation and failure of segmentation. They were demonstrated in an embryo of 13 mm and a fetus of 70 mm which were in the developmental stages of chondrification and early ossification, respectively. According to these observations, we conclude that most malformations of the vertebral body occur in the early stage of definitive vertebral body anlage formation.

*Key words:* embryology of spine; malformation of spine

Accepted 1.xi.80

Knowledge of the normal development of the vertebral column is essential to an understanding of congenital malformations of the vertebral body. Embryology of the human vertebral column has been described repeatedly (Bardeen & Lewis 1901, Bardeen 1905, Keyes & Compere 1932, Wyburn 1944, Sensenig 1949, and Peacock 1951). The study of Sensenig (1949) is the most extensive and best documented, and confirms the resegmentation theory which was proposed by Remak (1855) for the chick embryo. This concept to-day is almost universally accepted for all vertebral embryos (Flint 1977). Some authors, however, have taken issue with it. Verbout (1972) stated that each structure of the vertebra developed from the beginning in its final position and that a migration or shifting of sclerotomic halves, or a resegmentation of sclerotomes was unlikely to occur. It also must be emphasized that divergent interpretations of the early development are described in textbooks of Orthopaedics or Embryology (Patten 1968, Schmorl & Junghanns 1971, Hamilton et al. 1972, Rothman & Simeone 1975, and Epstein 1976).

The pathogenesis of congenital malformations of the vertebral body has been investigated and described by several authors (Junghanns 1937, Ehrenhaft 1943, Schmorl & Junghanns 1971, Rothman & Simeone 1975, Epstein 1976, and Tsou 1978). However, little attention has been paid to the relationship between malformation and the process of resegmentation, and the importance of resegmentation has never been described in detail. Congenital malformations of the vertebral body may be classified into two categories: 1) failure of formation and 2) failure of segmentation. As for failure of formation, Junghanns (1937) published schematic drawings of the normal and abnormal development of the vertebral body. The current concepts of the

0001-6470/81/030331-08 $02.50/0

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pathogenesis of congenital malformation of the vertebral body seem to be based on his work. However, it must be noted that he did not show any evidence proving his hypotheses, e.g. that the cartilaginous anlage of the vertebral body consists of two lateral halves. Junghanns did not comment on failure of segmentation. In fact, the mechanism remains controversial. Thus, the pathogenesis of both types of malformations is still open to question.

Over 200 human embryos and fetuses, ranging from 7 mm Crown-Rump length (5 weeks after fertilization) to 490 mm (full-term), were collected and studied histologically in an attempt to elucidate the pathogenesis of congenital malformation of the vertebral body. This study shows that both failures of formation and segmentation are present at the stage of chondrification, and that they most probably develop at an earlier stage. The purpose of this paper is to re-examine the normal development of the vertebral body, and to establish the importance of resegmentation in the pathogenesis of congenital malformations. The characteristics of the two principal types of anomaly in an embryo and in a fetus are reported in detail.

## MATERIAL AND METHODS

To illustrate normal development of the vertebral column, 7 embryos ranging from 7 mm Crown-Rump length (5 weeks after fertilization) to 13 mm (6 weeks) were selected. An embryo of 13 mm C-R length (6 weeks) demonstrates failure of formation, and a fetus of 70 mm C-R (11½ weeks) exemplifies failure of segmentation. Fertilization age was estimated according to the C-R length (Patten 1968) and confirmed by comparison of external morphological features (Hamilton et al. 1972).

All embryos were embedded in paraffin. The fetus of 70 mm was decalcified with 12 per cent E.D.T.A. Disodium salt for 4 weeks prior to embedding in paraffin. They were then cut in cross, sagittal or frontal planes. Sections were stained with Hematoxylin Phloxine Saffron, Toluidine Blue, Mann-Dominici or Goldner's Methods.

## RESULTS

### *Normal development*

The primitive segmentation of the sclerotomic tissue around the notochord is clearly demonstrated in an embryo of 7 mm C-R length. The intersegmental fissures which contain the intersegmental arteries constitute the borders of each segment. These sclerotomic segments correspond to the original somitic segments. Each sclerotomic segment is subdivided by the sclerotomic fissure into the cranial and caudal sclerotomic halves. In this early stage, however, there is little difference in cell density between

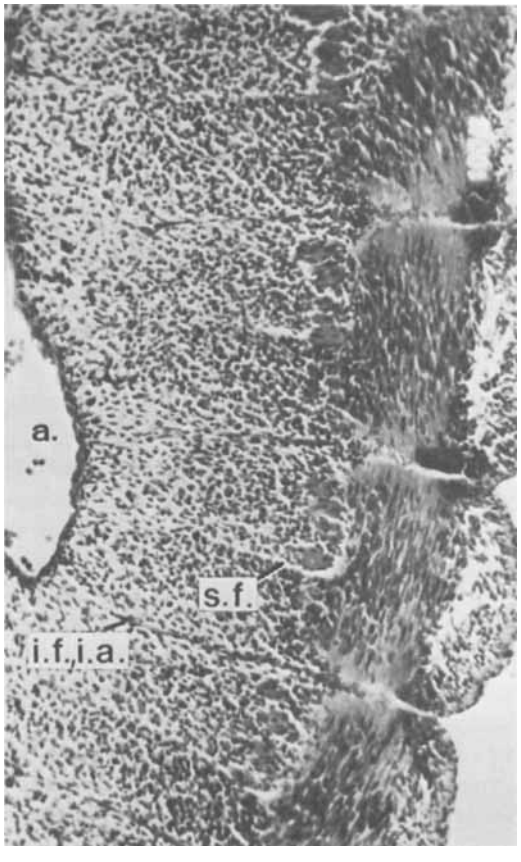


*Figure 1. An embryo of 7 mm C-R length. Sagittal section. The primitive sclerotomic segments and the sclerotomic halves. Note little difference in cell density between these halves at this stage. a.: aorta i.f.: intersegmental fissure, i.a.: intersegmental artery, s.f.: sclerotomic fissure.*

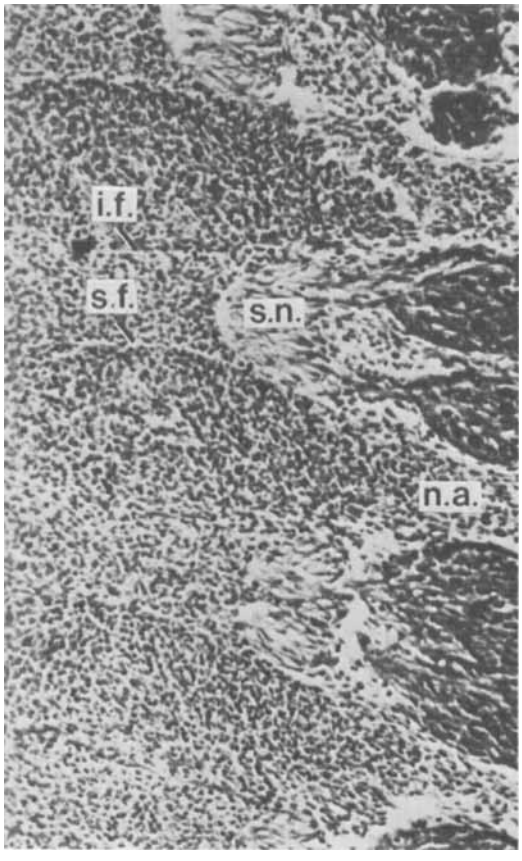


*Figure 2. An embryo of 8 mm. Sagittal section. Relationship between the loose-celled area, the dense-celled area, the spinal nerve and the rudiment of the neural arch is clearly shown. The spinal nerve is situated cranial to the neural arch in the primitive segment. i.f.: intersegmental fissure, s.f.: sclerotomic fissure, s.n.: spinal nerve, n.a.: rudiment of neural arch.*

these halves other than the gradual decrease in density from the area near the sclerotomic fissure to the intersegmental arteries both cranially and caudally (Figure 1).

In an embryo of 8 mm C-R length the sclerotomic column has loose- and dense-celled areas alternately arranged and definitely demarcated. However, neither the intersegmental arteries nor the sclerotomic fissures constitute the exact boundaries between loose-celled and dense-celled areas. Although the loose-celled area occupies mainly the cranial sclerotomic half of the segment, its cranial one-third extends cranially to the intersegmental artery and occupies also the caudal part of the caudal sclerotomic half of the adjacent segment. In the same manner, one-third of the dense-celled area spreads cranially into the cranial sclerotomic half whereas its caudal two-thirds are situated in the cranial part of the caudal sclerotomic half. The dense-celled area, which occupies mainly the caudal sclerotomic half, extends dorsolaterally to form the anlage of the neural arch. Therefore the neural arch is, in this stage, attached to the caudal portion of the primitive sclerotomic (or somitic) segment, and the spinal nerve of the same segment lies at the level of the cranial half of the same segment (Figure 2).

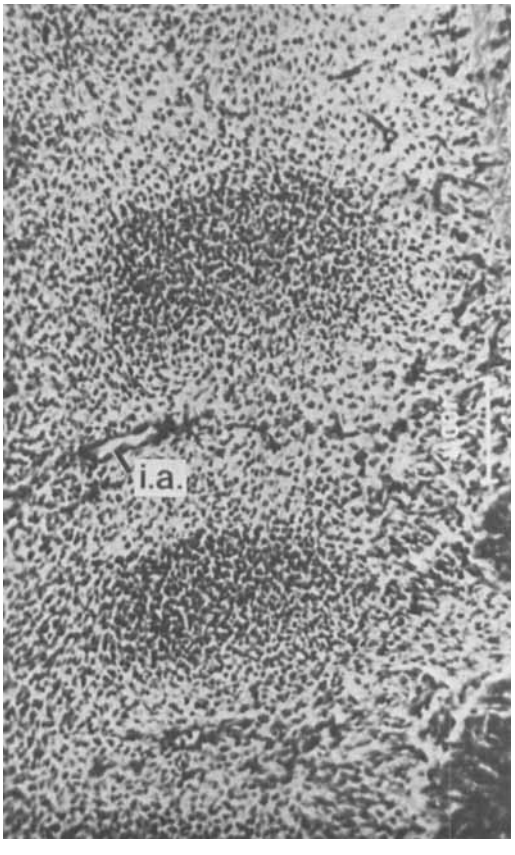


*Figure 3. An embryo of 8 mm. Sagittal section. The loose-celled and dense-celled areas are clearly seen alternately in this final stage of resegmentation. Note that the intersegmental artery is now situated in the middle portion of the loose-celled area. i.a.: intersegmental artery.*

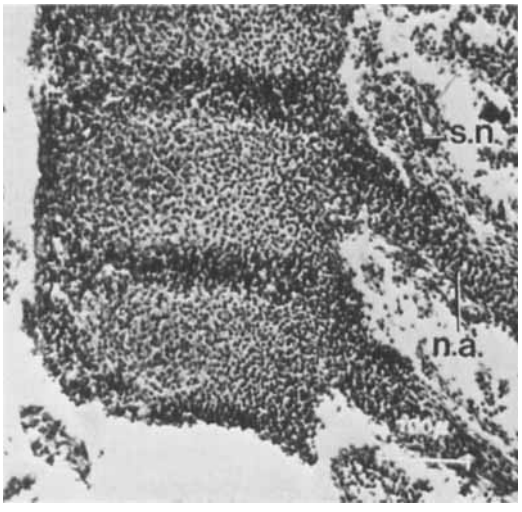


*Figure 4. An embryo of 12.5 mm. Sagittal section. The final relationship between the body, the neural arch and the spinal nerve. s.n.: spinal nerve, n.a.: neural arch.*

As the development progresses, the loose-celled area increases in height in its cranial part more than in its caudal part, causing a relative shift of the intersegmental artery to the middle of the loose-celled area. The dense-celled area in this stage shows three zones: the lighter cranial and caudal zones and the dense mid-zone. The lighter zones constitute an area of cell proliferation to supply the loose-celled area, thus contributing to the development of the definitive vertebral bodies. The dense mid-zone tends to change its position cranially and develops into the intervertebral disc anlage. Thus the loose-celled and dense-celled areas correspond to the anlagen of the vertebral body and the intervertebral disc, respectively (Figure 3).

In an embryo of 12.5 mm C-R length the vertebral body shows evidence of chondrification. The relationship between the vertebral body, the neural arch and the spinal nerve is now final. It no longer resembles the primitive segment divided by the intersegmental arteries. The neural arch, which still consists partially of undifferentiated mesenchymal cells, is attached to the cranial portion of the vertebral body, and the corresponding nerve (derived from the succeeding segment) is situated just caudal to it (Figure 4).

*Abnormal development*

a) *Failure of formation.* An embryo of 13 mm C-R length (6 weeks) cut sagittally shows a malformation of D10. The vertebral bodies are in the early stage of chondrification and the intervertebral disc spaces still consist of undifferentiated mesenchymal cells. At the level of D10 a wedge vertebra is noted in serial sagittal sections. The vertebral body is well formed on one side, but in its mid-portion the anterior part is decreased in height and the posterior part is an obscure small mass of precartilage. On the other side a thin plate of cartilaginous cells is seen. The area of the

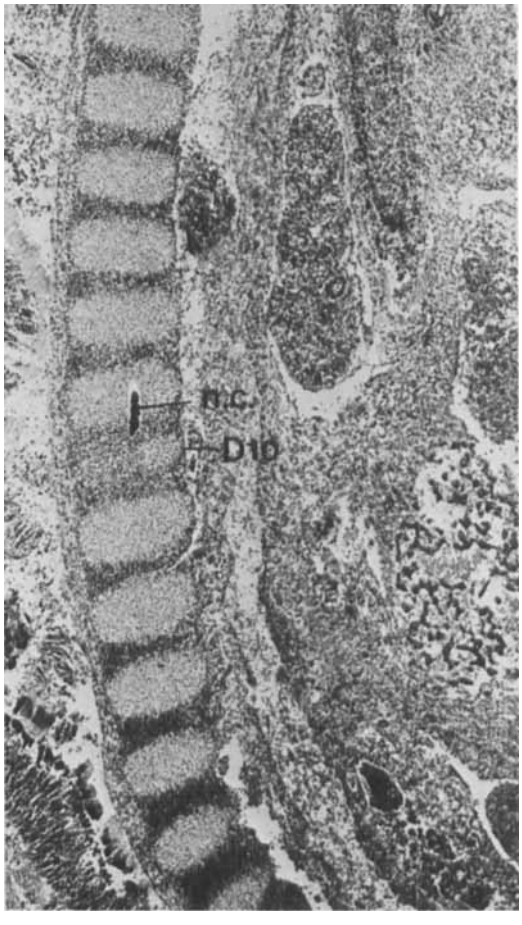


*Figure 5. An embryo of 13 mm. Mid-sagittal section. At the level of D10, the anterior part of the body is decreased in height and the posterior part is composed of an obscure mass of precartilage. n.c.: notochord.*

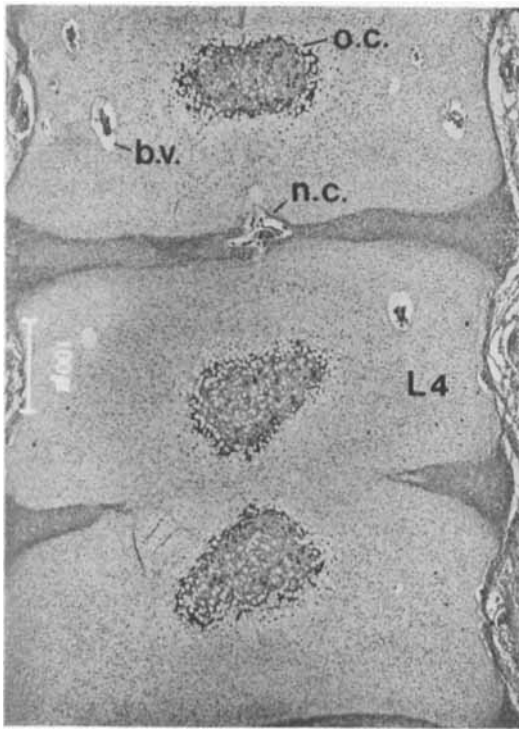


*Figure 6. A fetus of 70 mm. Frontal section. The segmentation has not taken place between L4 and L5. The unsegmented area consists of well-developed cartilaginous cells. o.c.: ossification centre, b.v.: blood vessel (cartilage canal), n.c.: notochord.*

defect is occupied by mesenchymal cells, as seen in the section of the mid-portion (Figure 5).

b) *Failure of segmentation.* Another malformation is shown in a fetus of 70 mm C-R length (11½ weeks) cut in a frontal plane. The segmentation between L4 and L5 has not taken place in the centrum, which is more evident anteriorly than posteriorly. The unsegmented area is occupied by well formed cartilage. At the periphery, remnants of the annulus fibrosus are conspicuous. Ossification has started in the centra of both L4 and L5, and ossification centres are closer to each other than normal (Figure 6).

## DISCUSSION

Remak (1855) discussed the early development of the vertebral column in chick embryos, pointing out the change of position of the neural arch and the spinal nerve. According to his observations, the rudiment of the neural arch is, at first, the dark zone continuous with the caudal dark area of the primitive vertebral segment, while the spinal nerve is situated just cranial to the neural arch, which corresponds to the level of the cranial light area. Later, in a definitive vertebra, the neural arch is attached to the cranial portion of the vertebral body and the nerve lies just caudal to the neural arch. Thus, he concluded that the vertebral column had changed its segmentation during the early development and he called it "new segmentation (Neue Gliederung)".

Many papers on the development of the vertebral column were produced thereafter (Bardeen & Lewis 1901, Bardeen 1905, Keyes & Compere 1932, Wyburn 1944, Sensenig 1949, and Peacock 1951). Sensenig (1949) carried out the last extensive study on which the current concepts of the vertebral development seem to be based. Our study largely confirms Sensenig's interpretation as well as the resegmentation theory.

As far as the cell differentiation of the definitive anlage of the vertebral body is concerned, the interpretation of Keyes & Compere (1932) seems to be well based. They considered that the formation of the vertebral body anlage was due to the more rapid differentiation of the mesenchymal cells nearer the intersegmental arteries than those further away because of a difference in the nutrient supply.

Our observations support this view entirely. We consider that the vertebral development at this stage is as follows: although the sclerotomic tissue shows, at first, the gradual decrease in cell density from the sclerotomic fissures to the intersegmental arteries cranially and caudally (Figure 7a), later the loose-celled area cranially and the dense-celled area caudally become evident. However, the intersegmental artery and the sclerotomic fissure, which limit the primitive sclerotomic segment and the sclerotomic halves of the segment, are not the exact boundaries of the loose-celled and dense-celled areas. The intersegmental artery is situated in the cranial one-third of the loose-celled area, and the sclerotomic fissure is situated in the cranial one-third of the dense-celled area. The dense-celled area extends

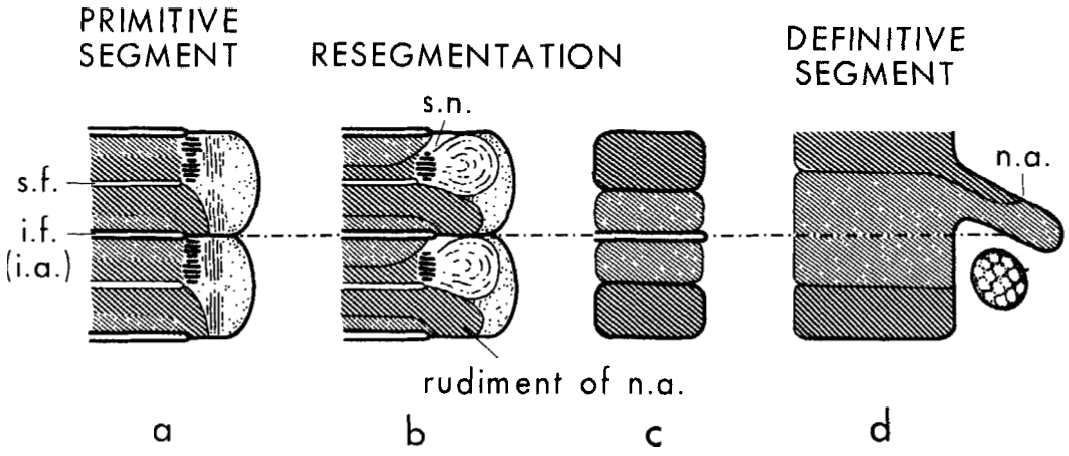


*Figure 7. Schematic drawings of the vertebral development. a; primitive segment, b and c; resegmentation d; definitive segment.*

dorsolaterally to form the rudiment of the neural arch, and the spinal nerve is situated at the level of the cranial sclerotomic half (Figure 7b). During further development, the loose-celled area increases in height in its cranial part more than in its caudal part. This augmentation in height seems to be due to the rapid differentiation and proliferation of cells taking place at the boundaries between the loose-celled and dense-celled areas. Cells are supplied with nutrition or stimulus for differentiation by the intersegmental artery, and their development progresses more rapidly at the cranial boundary than at the caudal boundary. This results in a cranial displacement of the dense-celled area (Figure 7c). Eventually the cranial part of the vertebral body is situated at the same level as the neural arch. The spinal nerve of the next segment, which remains in its original position, lies at the level of the caudal portion of the vertebral body (Figure 7d). Thus we strongly believe that the intersegmental artery is important in the formation of the vertebral anlage.

Congenital malformations of the vertebral body can be classified into two categories. One constitutes failure of formation and the other failure of segmentation. Failure of formation implies total or partial defects of the vertebral body such as aplasia of the body or hemivertebra, and also specific abnormalities in the shape of the vertebral body. On the other hand, failure of segmentation signifies unsegmented vertebrae. This can be partial, as in unilateral bar, anterior bar or posterior bar, or total, as in a block vertebra.

Junghanns (1937) made schematic drawings of the process of failure of formation. He believed that failure of formation was due to a lack of ossification. Recently Tsou (1978) described the embryology of congenital kyphosis, in which he concluded that most anomalies developed in the late stage of chondrification or in the stage of ossification. This theory seems to be more or less based on Junghanns' interpretation. However,

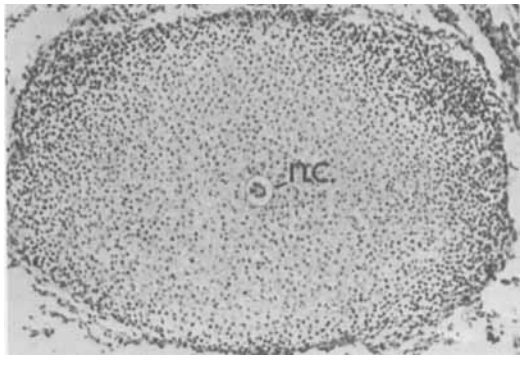


*Figure 8. An embryo of 13 mm. Cross section. The vertebral body consists of premature chondrocytes. Chondrification has started in a single cylindrical mass around the notochordal rod. There is no perichordal septum in the cartilaginous body. n.c.: notochord.*

our study does not support this view. Firstly, Junghanns' basic concept that the cartilaginous anlage of the vertebral body consists of two lateral halves was not observed in our study (Figure 8). Although Bardeen (1901, 1905) described two chondrification centres of the vertebral body divided by the perichordal septum, no descriptions of it can be found in the later literature (Keyes & Compere 1932, Wyburn 1944, Sensenig 1949, and Peacock 1951). Secondly, failure of formation is already present in the very early stage of chondrification in our embryo of 13 mm C-R length (6 weeks). It shows that a defect does not always correspond to the absence of half a body or a whole body. Failure of formation results from the abnormal differential growth of mesenchymal cells in the stage of resegmentation.

As for failure of segmentation, Tsou (1978) also mentioned that it was due to bony change of the fibrous annulus (osseous metaplasia of annulus fibrosus). In the case described here, failure of segmentation occurred before or in the very early stage of chondrification. It resulted from the absence of the intervertebral disc anlage.

The material presented here supports our view that both failure of formation and failure of segmentation must occur during the stage of resegmentation, or in the very early stage of chondrification.

## CONCLUSIONS

1. Resegmentation was observed to occur in the early stage of development.

2. The formation of the definitive vertebral body anlage is intimately related to the intersegmental artery.

3. Malformations are already present in the early stage of chondrification and most probably occur during resegmentation when the definitive vertebral body anlage is forming.

## ACKNOWLEDGEMENTS

The authors wish to thank Professor R. Narbaitz, Department of Anatomy, University of Ottawa, for his helpful criticism and suggestions, Dr. K. Sarkar for his assistance and advice, and Dr. D. P. Hill for his assistance in obtaining embryos. The authors also wish to thank Mrs. E. Duck, Mrs. L. Keng and Mrs. U. Portner for their technical help, Mr. S. Klosevych, Director of Department of Medical Communications of University of Ottawa, and his staff for preparation of illustrative material and Mrs. J. Patterson for preparation of the manuscript.

## REFERENCES

Bardeen, C. R. (1905) The development of the thoracic vertebrae in man. *Am. J. Anat.* **4**, 163–174.

Bardeen, C. R. & Lewis, W. H. (1901) Development of the limbs, body-wall and back in man. *Am. J. Anat.* **1**, 1–35.

Ehrenhaft, J. L. (1943) Development of the vertebral column as related to certain congenital and pathological changes. *Surg. Gynecol. Obstet.* **76**, 282–292.

Epstein, B. S. (1976) *The spine – A radiological text and atlas.* Lea and Febiger, Philadelphia.

Flint, O. P. (1977) Cell interactions in the developing axial skeleton in normal and mutant mouse embryos. In: *Vertebrale limb and somite morphogenesis.* (Ed. Ede, D. A. et al.) pp. 465–484, Cambridge University Press, Cambridge.

Hamilton, W. J., Boyd, J. D. & Mossman, H. W. (1972) *Human embryology.* Williams and Wilkins Co., Baltimore.

Junghanns, H. (1937) Die Fehlbildungen der Wirbelkörper. *Arch. Orthop. Unfallchir.* **38**, 1–24.

Keyes, D. C. & Compere, E. L. (1932) The normal and pathological physiology of the nucleus pulposus of the intervertebral disc. *J. Bone Joint Surg.* **14**, 897–938.

Patten, B. M. (1968) *Human embryology.* Third Edition, McGraw-Hill Book Co., New York.

Peacock, A. (1951) Observations on the pre-natal development of the intervertebral disc in man. *J. Anat.* **85**, 260–274.

Remak, R. (1855) *Untersuchungen über die Entwickelung der Wirbelthiere.* pp. 40–44. Verlag von G. Reimer, Berlin.

Rothman, R. H. & Simeone, F. A. (1975) *The spine.* W. B. Saunders Feb. First Edition, Philadelphia.

Schmorl, G. & Junghanns, H. (1971) *The human spine in health and disease.* Second American Edition, Grune and Stratton, New York.

Sensenig, E. C. (1949) The early development of the human vertebral column. *Contr. Embryol. Carnegie Inst.* **33**, 21–41.

Tsou, P. M. (1978) Embryology of congenital kyphosis. *Clin. Orthop.* **128**, 18–25.

Verbout, A. J. (1972) The process of segmentation in the vertebral column. *Acta Morph. Neel. Scand.* **9**, 384–385.

Wyburn, G. M. (1944) Observations on the development of the human vertebral column. *J. Anat.* **78**, 94–102.

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